LES TANINS DE *KALMIA ANGUSTIFOLIA* COMME AGENTS DE CONTRÔLE DE LA DISPONIBILITÉ DES NUTRIMENTS DANS LES PESSIÈRES BORÉALES

par

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Cette thèse porte sur les mécanismes par lequel des plantes qui produisent une litière riche en composés phénoliques et tanins condensés réduisent la disponibilité des nutriments, surtout l'azote, dans le sol. Les mécanismes étudiés sont la séquestration de l'azote dans des complexes protéines-tanins, le changement des formes d'azote disponible dans le sol, l'inhibition des enzymes du sol et, finalement, la réduction de la disponibilité des nutriments par une végétation riche en tanins selon le type de site et perturbation. Ces mécanismes ont été étudiés dans le contexte de l'envahissement des parterres de coupe de peuplement d'épinette noire par une éricacée, le *Kalmia* (*Kalmia angustifolia*), qui est une plante qui contient de fortes concentrations en tanins condensés dans sa litière et qui est souvent associée à une diminution de la fertilité des sites.

En premier lieu, je me suis intéressé à la séquestration de l'azote sous forme de complexes protéines-tanins comme mécanisme de contrôle de la disponibilité de l'azote suivant l'envahissement par le *Kalmia.* J'ai comparé la capacité de formation de complexes protéines-tanins de *Kalmi*a et d'épinette, j'ai ajouté des tanins et des complexes protéines-tanins dans de l'humus pour mesurer leurs minéralisations par les microorganismes, et finalement, j'ai comparé la croissance de mycorhizes sur des milieux contenants des tanins et des complexes protéines-tanins. Les résultats indiquent que, par unité de masse, les tanins de *Kalmia* étaient plus efficaces pour précipiter les protéines et que les complexes protéines-tanins étaient ni minéralisés ni utilisés par les microorganismes de l'humus. Finalement, les mycorhizes d'épinette avaient une croissance très faible dans les milieux contenant des complexes protéines-tanins relativement aux mycorhizes du *Kalmia.* Nos résultats suggèrent que la présence de *Kalmia* sur un site augmente la quantité d'azote séquestré sous forme de complexes protéines-tanins, et que ceci lui conférerait un avantage envers l'épinette.

En deuxième lieu, je me suis intéressé au concept que l'augmentation de litière riche en composés phénoliques sur des sites infertiles en fin de succession entraîne une augmentation du ratio de l'azote organique dissous (DON) par rapport à l'azote inorganique dissous (DIN) dans le sol. Il est proposé que cette augmentation du ratio soit due par la formation de complexes protéines-tanins et un ralentissement de la minéralisation des litières riches en composés phénoliques. Compte tenu que d'autres facteurs peuvent influencer ce ratio tel que l'activité microbienne et l'immobilisation subséquente de nutriments, j'ai réalisé une expérience dans laquelle j'ai ajouté des proportions grandissantes de litières de *Kalmia* par rapport à celle d'épinette noire dans de l'humus et j'ai mesuré le ratio DON :DIN, ainsi que l'activité microbienne. Nos résultats suggèrent la formation de complexes protéines-tanins par la litière de *Kalmia*, puisque l'activité microbienne ainsi que l'azote minéral n'ont pas augmentés même si la litière de *Kalmia* contenait deux fois plus d'azote que la litière d'épinette. Cependant, le ratio DON : DIN n'a augmenté en fonction de la proportion de *Kalmia* qu'à seulement une des dates d'incubation, ce qui suggère que ce ratio n'est pas un bon indice de l'évolution vers des communautés riches en tanins condensés.

En troisième lieu, je me suis intéressé au concept de l'inhibition enzymatique par les tanins relâchés par la litière comme mécanisme pouvant expliquer la faible disponibilité des nutriments dans des sols envahis par le *Kalmia*, et de même, son contrôle sur les processus de l'écosystème. Pour ce faire, j'ai mesuré l'activité d'enzymes du sol en présence de tanins purifiés, en présence de litière de *Kalmia* et d'épinette, et finalement, en relation avec le pourcentage de recouvrement de *Kalmia* sur le terrain. Les résultats ont montrés que les tanins inhibent l'activité des enzymes du sol et que cette inhibition est proportionnelle à la concentration de tanins. Une diminution de l'activité de certains enzymes a été mesurée suivant l'augmentation de litière de *Kalmia* et son pourcentage de recouvrement sur le terrain. Globalement, ces résultats supportent l'hypothèse que l'inhibition enzymatique par les tanins de la litière de *Kalmia* est un mécanisme important qui contrôle les processus de l'écosystème.

En quatrième lieu, compte tenu que les concentrations en tanins et composés phénoliques variaient avec les conditions de fertilité et de luminosité du site, j'ai voulu comparer la disponibilité des nutriments dans des sols associés au *Kalmia* et à l'épinette noire de forêts fermées et de parterres de coupes à lichens et à mousses. Nos résultats indiquent des différences entre les types de sites, mais, peu importe le type de site, le *Kalmia* diminuait les disponibilités des formes d'azotes (DIN et DON) et du carbone pour les organismes du sol relativement à l'épinette noire, quoique qu'il les diminuait un peu moins dans les forêts fermées, possiblement à cause d'une litière de meilleure qualité. Cette étude démontre qu'il est important de tenir compte du recouvrement de *Kalmia* avant coupe forestière, car ce dernier diminue la disponibilité des nutriments du sol même sous la canopée.

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INTRODUCTION

La forêt boréale est un milieu dynamique qui recouvre une grande partie de la surface terrestre. Au Québec, la forêt boréale occupe une superficie de 551 400 km^2 et est une ressource économique très importante. Depuis l'adoption de la Stratégie de protection des forêts en 1994, la gestion forestière au Québec mise d'abord et avant tout sur la régénération naturelle. Les méthodes de récolte ont ainsi évolué de façon à protéger autant que possible la régénération déjà en place au moment de l'intervention. De ce fait, le principal type de coupe utilisé dans la forêt boréale est la Coupe avec protection de la régénération et des sols (CPRS). La coupe CPRS est la coupe de tous les arbres adultes d'une forêt, selon des techniques qui permettent de protéger les petits arbres en croissance sur les aires de récolte et de minimiser l'impact négatif des opérations forestières sur l'état des sols (Gouvernement du Québec, 2003). La CPRS représente en général plus de 50% (allant jusqu'à 85% dans certaines régions) de la superficie de coupe dans la forêt boréale (Gouvernement du Québec, 2007). Ce type de coupe est largement utilisé dans les peuplements d'épinette noire (*Picea mariana* (Mill.) BSP) de la forêt boréale. Cependant, il arrive dans de nombreux cas que le rendement prévu de la croissance, soit de marcotte ou de semis, soit largement réduite sur certains de ces sites, même si les sites étaient bien classés au niveau du potentiel relié à la fertilité. Sur nombres de ces sites, on y note une densité importante d'éricacées sur les parterres de coupe, qui serait alors responsable de la faible croissance des épinettes (Mallik, 1995 ; Thiffault et Grondin, 2003 ; Titus *et al.*, 1995).

Les éricacées, dont le *Kalmia angustifolia,* le *Rhododendron groenlandicum* et *Vaccinium sp.*, se retrouvent dispersés dans l'étage arbustif de la forêt avant coupe. Suivant l'ouverture de la canopée, il y a une prolifération et un envahissement progressif par les éricacées par voie de reproduction végétative (Titus *et al.*, 1995) et ensuite, selon l'ampleur et la rapidité de

l'envahissement, une croissance diminuée, voir arrêtée (growth check) des conifères et une production nette diminuée du site en résulte (Ruel *et al.*, 2004). Une croissance stagnante des conifères a également été observée dans d'autres communautés de conifères-éricacées à travers le monde, tel qu'avec *Pinus sylvestris-Empetrum hermaphroditum* Hagerup (Nilsson *et al.*, 1993 ; Nilsson et Zackrisson, 1992) en Scandinavie, *Thuja plicata Donn* ex D. – *Gaulteria shallon* Pursh sur la côte ouest (Fraser *et al.*, 1995 ; Fraser *et al.*, 1993 ; Prescott, 1996), *Pinus sylvestris-Calluna vulgaris* (L.) Hull (Mallik, 1995) et *Picea abies – Vaccinium myrtillus* L. (Gallet, 1994). Et de ce, plusieurs études ont été réalisées pour comprendre les mécanismes en cause (Voir Mallik, 2003 ; Thiffault et Grondin, 2003). Trois principaux mécanismes ont été proposés ; soit l'allélopathie (tout effet direct ou indirect, positif ou négatif, d'une plante sur une autre plante, par le biais de composés biochimiques libérés dans l'environnement (Rice 1984)), soit la compétition directe pour les ressources, soit la modification de la disponibilité des nutriments du sol autrement que par l'acquisition des ressources par la formation d'un humus récalcitrant à la décomposition (voir ici-bas). Bien que ces trois mécanismes ne soient pas mutuellement exclusifs, plusieurs études démontrent une diminution de la quantité de nutriments disponibles et extractibles du sol, dont l'azote minéral (ex., Damman, 1971) et subséquemment, une faible acquisition de N par les conifères dans des sols d'éricacées suggérant une séquestration des nutriments dans un humus récalcitrant (Bradley *et al.*, 1997). Compte tenu que l'azote est souvent l'élément limitant dans la forêt de conifère boréale pour la croissance des arbres (Pastor *et al.*, 1987), il est important de comprendre les mécanismes par lesquels sa disponibilité diminue suivant l'envahissement des éricacées sur les parterres de coupe de différents types de peuplement. De ce fait, des évidences suggèrent que la litière des éricacées, qui est riche en composés phénoliques, dont les tanins condensés, serait responsable de la diminution de la disponibilité du N, et pourrait procurer un avantage aux éricacées (Kraus *et al.*, 2003a ; Mallik, 2003 ; Mallik et Inderjit, 2001).

L'importance des tanins dans les fonctions des écosystèmes et dans le cycle des nutriments est reconnu depuis longtemps (Handley, 1961 ; Horner et Gosz, 1988), et des études récentes l'ont démontrées (Bradley *et al.*, 2000 ; Hattenschwiler et Vitousek, 2000 ; Northup *et al.*, 1998 ; Preston, 1999 ; Schimel *et al.*, 1998 ; Schimel *et al.*, 1996). Dans les espèces ligneuses, on retrouve des concentrations typiques de composés phénoliques et de tanins condensés variant de 1 à 30 % du poids sec des feuilles (Kraus *et al.*, 2004a ; Mansfield *et al.*, 1999 ; Preston, 1999 ; Yu et Dahlgren, 2000), et les éricacées ont typiquement des concentrations très élevées (Gallet et Lebreton, 1995). Les tanins, qui sont des molécules riches en carbone, sont divisés dans deux classes majeures, soit les tanins hydrolysables et les tanins condensés. Les tanins hydrolysables sont des esters de l'acide gallique ou de l'acide ellagique liés à un monosaccharide comme le glucose (Figure 1a). Les tanins condensés sont des polymères formés de monomère de flavon-3-ol liés entre eux par des liens Carbone-Carbone qui peuvent posséder différentes stéréochimies, longueurs de chaîne, et différents groupement OH sur l'anneau B des monomères de flavon-3-ol (Figure 1b). Les monomères qui forment les tanins peuvent être regroupés selon le nombre de groupement OH retrouvé sur l'anneau B. Lorsque le monomères possède deux groupement OH (anneau B dihydroxy), on le nomme procyanidine (PC) alors que lorsqu'il en possède trois (anneau B trihydroxy) on le nomme prodelphinidine (PD). Les monomères peuvent également avoir différentes stéréochimies aux carbones C-2-C3 (cis ou trans). Le lien entre les monomères se fait typiquement par des liaisons C-4→C-8, mais des liaisons C-4→C-6 sont également possibles. De plus, le nombre de monomères liés entre eux peut varier, créant ainsi des tanins de différentes longueurs de chaînes. Ces différences structurales font qu'ils peuvent réagir différemment dans l'environnement (Kraus *et al.*, 2003b). Dans le *Kalmia* et l'épinette noire, on ne retrouve que des tanins de types condensés, et des études antérieures ont démontré que leurs structures étaient différentes (Bradley *et al.*, 2000 ; Lorenz et Preston, 2002 ; Nierop *et al.*, 2005 ; Preston *et al.*, 1997). Suivant la sénescence des feuilles et des racines ainsi que le lessivage par la pluie, de grandes quantités de composés phénoliques pénètrent dans le sol. Ces composés peuvent jouer un rôle dans le cycle des nutriments et la décomposition des litières et dans la disponibilité de l'azote de plusieurs façons (Hattenschwiler et Vitousek,

2000 ; Kraus *et al.*, 2003a). Premièrement, ils pourraient avoir des effets toxiques sur les microorganismes et autres organismes décomposeurs (Scalbert, 1991). Deuxièmement, ils pourraient séquestrer les protéines dans des complexes protéines-tanins résistants à la décomposition. Troisièmement, ils pourraient se lier à d'autres composés, tel que la cellulose, et les protégeant d'attaques microbiennes. Quatrièmement, ils pourraient inhiber les enzymes, et cinquièmement, ils pourraient engendrer une immobilisation du N par les microorganismes qui utilisent les tanins comme source de carbone et d'énergie (Kraus *et al.*, 2003a et réf.). Il est clair que plusieurs de ces mécanismes

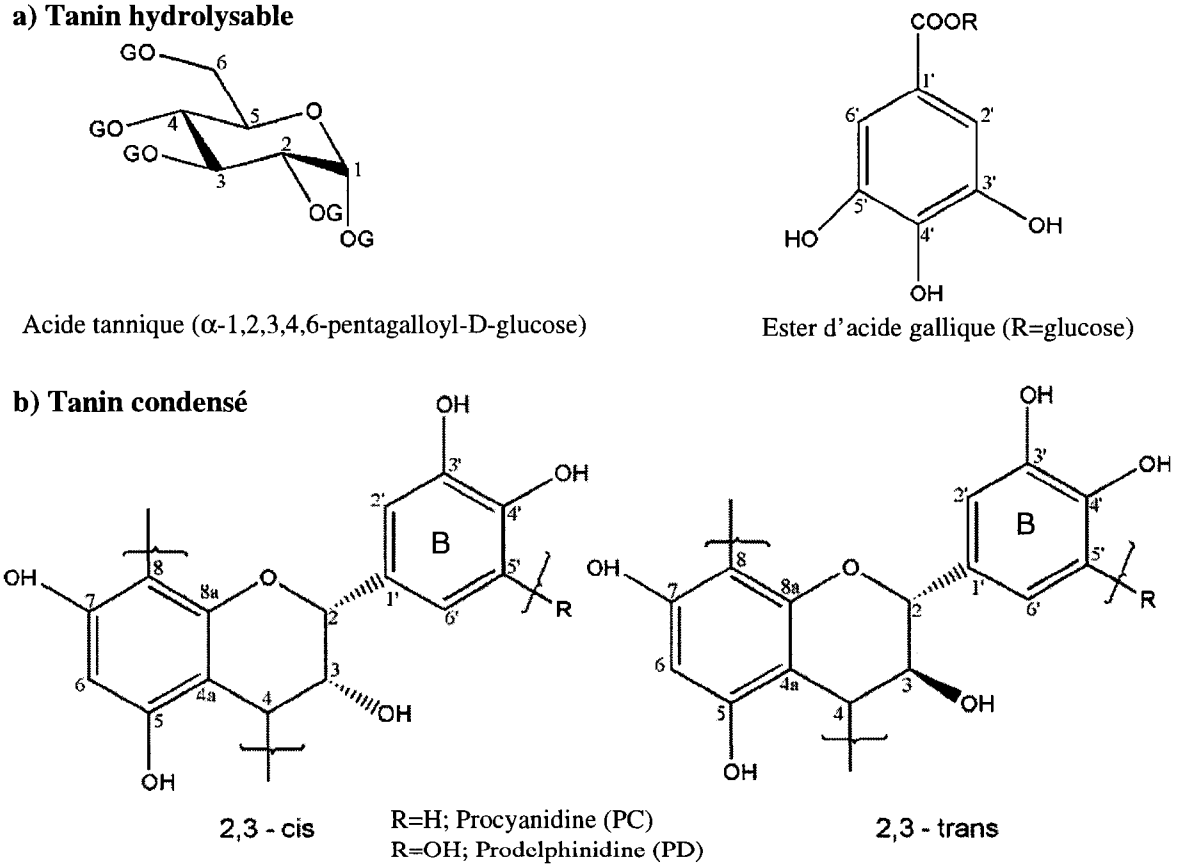

Figure 1. Structure de a) tanin hydrolysable et b) tanin condensé (adaptée de Kraus *et al.* 2003b)

pourraient agir en concomitance, mais la caractéristique principale qui défini un tanin est sa capacité de précipiter des protéines et ainsi de modifier les cycles biogéochimiques (Hattenschwiler et Vitousek, 2000 ; Kraus *et al.*, 2003a ; Kraus *et al.*, 2003b).

Il est connu depuis longtemps que l'association des tanins avec la peau d'animaux (collagène) donne du cuir, un matériel résistant aux intempéries et à la décomposition (*Kraus et al.*, 2003a). De la même façon, il a été suggéré que les tanins pourraient se lier aux protéines et former des complexes récalcitrants à la décomposition dans les sols. Les complexes peuvent se former dans les feuilles/racines lors de la sénescence, ou bien dans le sol suivant le lessivât des tanins des feuilles/racines. Les tanins forment des complexes avec les protéines par des liaisons de type hydrogène et par des effets hydrophobiques (Hagerman *et al.*, 1998 ; Kraus *et al.*, 2003a). Plusieurs facteurs font varier la capacité de précipiter les protéines par les tanins, tel que le pH (Martin et Martin, 1983), les concentrations en tanins et en protéines (Spencer *et al.*, 1988) et la structure des tanins et des protéines (de Freitas et Mateus, 2001 ; Kraus *et al.*, 2003b). Les diverses études démontrent que les complexes protéines-tanins sont beaucoup plus résistants à la décomposition microbienne que de simples protéines (Benoit *et al.*, 1968 ; Davies *et al.*, 1964 ; Handley, 1954 ; Handley, 1961 ; Howard et Howard, 1993 ; Lewis et Starkey, 1968 ; Mutabaruka *et al.*, 2007 ; Wu *et al.*, 2003). En plus d'avoir différentes capacités à précipiter les protéines, certains tanins peuvent former des complexes protéines-tanins qui sont plus difficiles à dégrader que d'autres. Par exemple, Handley (1961) a démontré que les complexes formés avec des extraits de la bruyère (*Calluna vulgaris)*, une éricacée, étaient plus résistants que des complexes formés avec d'autres tanins. Howard et Howard (1993) ont démontré également que la libération de N-NH_4 de complexes protéines-tanins, en culture liquide, variait selon l'origine des tanins, et que la capacité de précipitation n'était pas nécessairement liée à la résistance à la dégradation. De plus, la dégradation et la minéralisation des complexes protéines-tanins variaient en fonction des communautés microbiennes présentes et des différents types de mycorhizes (Bending et Read, 1996 ; Mutabaruka *et al.*, 2007 ; Wu *et al.*, 2005).

Les mycorhizes sont impliqués dans l'acquisition des nutriments pour la plupart des plantes terrestres (Smith *et a*l,. 1997). Généralement, les plantes vasculaires absorbent des nutriments inorganiques de la solution de sol ou de simples molécules organiques (ex., acides aminées), mais la présence de mycorhizes peut leur donner accès à des sources de nutriments additionnels par l'activité enzymatique des hyphes ou par d'autres modifications chimiques ou physiques de la rhizosphère (Marschner, 1995). Dans la forêt boréale, les principaux types de mycorhizes sont des ectomycorhizes (ECMs) et des mycorhizes éricoïdes (ERCs) (Read *et al.*, 2004 ; Read et Perez-Moreno, 2003). Les ECMs sont majoritairement des champignons basidiomycètes, mais on retrouve également des ascomycètes, et ce composent de nombreuses espèces différentes. Chez les ECMs, les hyphes occupent l'espace intercellulaire et forme un réseau (*réseau de Hartig*) et entourent les racines en formant un manteau de mycélium. Les ERCs impliquent majoritairement des champignons ascomycètes, mais également des basidiomycètes, et représentant peu d'espèces différentes. Chez les ERCs, les hyphes prolifèrent à l'intérieur des cellules épidermales des racines et forment des pelotons (Read *et al.*, 2004). Ces deux types de mycorhizes possèdent certaines propriétés saprophytes et peuvent dégrader une multitude de composés organiques. Les ERCs possèdent diverses enzymes (Bending et Read, 1997 ; Burke et Cairney, 2002) qui leur donnent une bonne habileté pour dégrader des molécules complexes dans les sols et satisfaire leur besoin en carbone et nutriments. Dans le cas des ECMs, l'activité saprophyte serait également présente, mais généralement plus faible que pour les ERC (Bending et Read, 1997 ; Read *et al.*, 2004 ; Read et Perez-Moreno, 2003). De ce fait, la plupart de études réalisées montrent que la plupart des ERC sont capables de croître sur des complexes protéines-tanins alors que des ECM sont incapables (Bending et Read, 1996 ; Wu *et al.*, 2003). De ces études, les auteurs suggèrent que la production de complexes protéines-tanins par les éricacées serait une adaptation leur permettant de réduire le N disponible pour les autres plantes, et il y aurait une rétroaction positive entre la production de composés phénoliques des plantes en milieux pauvres et un avantage compétitif de ces plantes envers les autres plantes du milieu. Cependant, ces études ont toutes utilisées des complexes protéines-tanins formés avec de l'acide tannique, un tanin hydrolysable générique, qu'on ne retrouve pas dans ces milieux,

qui réagit différemment à la plupart des tests de précipitation et est supposément plus labile que la plupart des tanins condensés (Kraus *et al.*, 2003a ; Kraus *et al.*, 2003b ; Mutabaruka *et al.*, 2007).

De fortes concentrations de composés phénoliques ont été mesurées dans les communautés végétales de fin de succession se trouvant sur des sites infertiles et acides à travers le monde (Northup *et al.*, 1995a ; Northup *et al.*, 1998 ; Wardle *et al.*, 2004 ; Wardle *et al.*, 1997). Il a été proposé que ces fortes concentrations dans les litières augmentent la fitness de ces plantes à cause de l'effet de ces composés phénoliques sur la minéralisation des litières et le relâchement des différentes formes solubles d'azote du sol (i.e., organique (DON) versus minérale (DIN)), et subséquemment leur ratio respectif (DON : DIN) (Northup *et al.*, 1995a ; Northup *et al.*, 1998 ; Northup *et al.*, 1995b). Northup et al (1995b) ont trouvé que suivant un gradient de fertilité, il y avait une corrélation négative entre les concentrations de composés phénoliques dans les aiguilles du pin Bishop (*Pinus muricata* D. Don) et le relâchement de l'azote inorganique lors de la décomposition, mais une corrélation positive avec le relâchement de DON. Une augmentation du ratio DON:DIN favoriserait les espèces qui sont capables d'absorber l'azote sous forme organique par rapport à celle qui ne sont pas capables, ou moins efficaces. Ils suggèrent que ce serait à cause de la formation de complexes protéines-tanins qui se trouverait en solution du sol et qui serait difficilement dégradables, et que ceci procurerait un avantage aux plantes produisant ces tanins. Cependant, des études récentes n'ont pas montré de lien consistant avec le ratio DON :DIN dans le sol et la quantité de tanins (Holub et Lajtha, 2004 ; Kanerva *et al.*, 2006). Le ratio DON : DIN peut également varier si les organismes du sol immobilisent de l'azote suivant l'ajout de litière contenant de fortes concentrations en composés phénoliques, qui agi alors comme source de carbone (Kraus *et al.*, 2004b). Donc, il est envisageable qu'il y aura une augmentation d'azote organique dissous suivant l'envahissement par des plantes contenant de fortes concentrations de composés phénoliques.

La dépolymérisation de la matière organique du sol par les enzymes extracellulaires microbiennes, avec subséquemment le relâchement de nutriments disponibles pour les plantes, est un facteur clé dirigeant les cycles biogéochimiques (Schimel et Bennett, 2004) et peut être l'étape limitante de la décomposition (Schimel et Weintraub, 2003 ; Sinsabaugh et Moorhead, 1994). L'activité des enzymes peut être influencée par les propriétés abiotiques du sol, les organismes présents et leurs activités, le couvert végétal, les entrées de produits de lixiviation et la présence d'inhibiteurs ou d'activateurs (Andersson *et al.*, 2004 ; Dilly et Munch, 1996 ; Fioretto *et al.*, 2000 ; Kourtev *et al.*, 2002). Certaines études ont démontré que des composés phénoliques ou des extraits de plantes pouvaient inhiber l'activité de certains enzymes (Bell *et al.*, 1965 ; Benoit et Starkey, 1968 ; Goldstein et Swain, 1965), alors que d'autres n'ont pas démontré d'effet sur l'activité enzymatique lors de l'ajout de composés organiques riches en composés phénoliques (Fierer *et al.*, 2001 ; Madejon *et al.*, 2001). Compte tenu de l'importance des enzymes du sol, et que ceux-ci sont des protéines, il va de soi que les tanins relâchés par une plante pourraient diminuer la disponibilité des nutriments indirectement par l'inhibition des enzymes du sol.

La production et les concentrations de tanins condensés retrouvées dans les feuilles et racines varient selon les conditions environnementales et nutritionnelles d'un site (Koricheva *et al.*, 1998 ; Kraus *et al.*, 2003a). Compte tenu que ce sont des composés secondaires riches en carbone, si la croissance de la plante est limitée par un manque de nutriments (ex. N) par rapport à la lumière, il y aura un excès de carbone dans la plante. Alors, la plante augmenterait l'allocation de composés riches en carbone, tel que des composés phénoliques et des tanins plutôt que des protéines (Bryant *et al.*, 1983). Également, si peu de nutriments sont disponibles, il y a plus de contraintes sur la croissance que sur la photosynthèse, amenant donc une accumulation en hydrates de carbone servant alors à la production de composés phénoliques (Lambers *et al.*, 1998). Donc, dans des conditions de faible luminosité, comme sous la canopée forestière, il est attendu que la production de tanins condensés sera limitée et que par conséquent, une moins grande concentration entrera dans le sol par rapport à des

conditions de forte luminosité, comme sur les parterres de coupes (Heath et Arnold, 1966 ; Iason et Hester, 1993 ; Koricheva et al., 1998). De plus, l'effet de cette litière riche en composés phénoliques sur les processus du sol et la décomposition varie selon des caractéristiques biotiques et abiotiques d'un site, tel que la texture du sol, le drainage, et la végétation accompagnatrice (Castells et Penuelas, 2003 ; Castells *et al.*, 2004 ; Thomas et Prescott, 2000). Par exemple, les éricacées de la forêt boréale se trouvent sur divers types de site, tel que des sites dont le couvre sol est composé de mousses et d'autres sites où le couvre sol se composent de lichens. Ces deux types de végétaux non-vasculaires modifient différemment les processus du sol, tel que la décomposition des litières, la température du sol et l'accumulation de matière organique (Cornelissen *et al.*, 2007; Lamontagne, 1998 ; Nilsson et Wardle, 2005 ; Sedia et Ehrenfeld, 2003 ; Sedia et Ehrenfeld, 2005; Sedia et Ehrenfeld, 2006; Wardle *et al.*, 2003). En général, ces études démontrent que moins d'accumulation de matière organique se produit sous les lichens et un plus faible taux de décomposition des litières de plantes vasculaires associées à un couvre sol de lichen que de mousse. Donc, une plante sur un site couvert de lichen devrait avoir moins de nutriments disponibles et produire une litière avec des fortes concentrations en composés phénoliques qui affectera plus les processus du sol que sur des sites recouverts de mousses.

Ainsi, l'objectif principal de cette thèse porte sur les mécanismes de réduction de la disponibilité des nutriments, surtout l'azote, par les tanins du *Kalmia angustifolia.* Compte tenu que l'épinette noire possède également des tanins condensés, et qu'il se retrouve dans le même milieu, il est important de comparer l'effet de ces derniers avec ceux du *Kalmia.* On émet l'hypothèse générale que les tanins du *Kalmia* sont plus efficaces pour réduire la disponibilité des nutriments du sol que les tanins d'épinette noire et que ceci procure un avantage compétitif au *Kalmia.* Dans le premier chapitre, nous testons l'hypothèse que la formation de complexes protéines-tanins est un mécanisme important de la séquestration de l'azote dans le sol, et que cela procure un avantage à *Kalmia* qui possède des mycorhizes éricoïdes. Dans le deuxième chapitre, l'objectif était de déterminer si une augmentation de

litière de *Kalmia* riche en tanins inhibent les taux de minéralisations, réduit l'activité microbienne dans le sol et augmentent le ratio DON : DIN. Dans le troisième chapitre, nous testons l'hypothèse qu'une diminution de la disponibilité des nutriments suivant l'envahissement par le *Kalmia* est en partie due à une inhibition de l'activité des enzymes du sol. Dans le dernier chapitre, l'objectif était de déterminer comment la disponibilité de l'azote, du carbone, ainsi que la décomposition de litière étaient réduites par le *Kalmia* dans des forêts et des parterres de coupe de lichen et de mousse. Finalement, des résultats préliminaires montrant la variation des concentrations en tanins condensés et composés phénoliques dans les feuilles et racines de *Kalmia* en fonction de la luminosité et de la fertilité sont présentés en annexe.

CHAPITRE I

LA SÉQUESTRATION DE L'AZOTE SOUS FORME DE COMPLEXES PROTÉINES-TANINS AMÉLIORE L'HABILITÉ COMPÉTITIVE DU *KALMIA* RELATIVEMENT À L'ÉPINETTE NOIRE

Référence: Joanisse, G.D., Bradley, R.L., Preston, C.M., Bending, G.D. Sequestration of soil-N as tannin-protein complexes may improve the competitive ability of sheep laurel (*Kalmia angustifolia*) relative to black spruce (*Picea mariana*). (soumis à New Phytol.)

Le mécanisme le plus souvent proposé pour expliquer la faible disponibilité de l'azote dans les parcelles envahies par les éricacées, tel le *Kalmia*, est par la formation de complexes protéines-tanins résistants à la dégradation dans les sols. Il est également suggéré que ceci lui procurerait un avantage compétitif à cause de ces mycorhizes éricoïdes potentiellement capable de dégrader de tels composés organiques complexes. Cependant, la formation de complexes protéines par les tanins de *Kalmia* a toujours été inférée indirectement et n'avait pas encore été vérifiée. De plus, puisque l'épinette noire possède également des tanins condensés dans ses aiguilles, et qu'ils sont structurellement différents de ceux du *Kalmia*, il était donc important de comparer la formation et la dégradation de complexes protéines-tanins provenant de ces deux espèces. L'objectif principal de ce papier était de vérifier l'hypothèse que la formation de complexes protéines-tanins par les tanins du *Kalmia* est un mécanisme lui conférant un avantage vis-à-vis l'épinette noire. Plus précisément, le manuscrit présente des évidences que (1) les tanins purifiés et les extraits de feuilles de *Kalmia* sont plus efficaces pour former des complexes protéines-tanins que ceux de l'épinette (2) peu importe l'origine des tanins et des complexes protéines-tanins, ils ne sont que très peu

minéralisés dans les sols, donc peu utilisé comme source de C et de N et (3) et que les mycorhizes communément associés au *Kalmia* avaient une meilleure croissance sur des complexes protéines-tanins que des mycorhizes communément associés à l'épinette noire. Donc cette étude a permis d'avancer notre compréhension du mécanisme d'action des tanins condensés relâchés par le *Kalmia* et donne des évidences que le *Kalmia* à un avantage compétitif sur l'épinette noire en ayant accès à des sources d'azotes non directement disponible pour l'épinette.

J'ai élaboré et réalisé les expériences de laboratoire, ainsi que l'analyse des spectres RMN, et ma participation à la rédaction du manuscrit fut importante. La Dr. Caroline Preston a caractérisé les tanins condensés du *Kalmia* et d'épinette que j'ai purifiés et que j'ai utilisés dans les deux premières expériences, en plus d'avoir participé à la correction et proposer de nombreuses suggestions lors de la rédaction de l'article. Le Dr. Gary Bending m'a accueilli dans son laboratoire et m'a donné une formation sur la manipulation des mycorhizes, en plus d'avoir révisé le manuscrit. Le Dr. Bill Parsons a également révisé l'orthographe et la grammaire anglaise d'une première version de ce manuscrit. La rédaction du manuscrit a été réalisée avec la collaboration de mon directeur, Dr. Robert Bradley.

Summary

- The role of litter tannins in controlling soil N cycling may explain the competitive ability of *Kalmia* relative to black spruce, although this has not been demonstrated experimentally.

- We compared the protein-precipitation capacities of purified tannins and leaf extracts from *Kalmia* and black spruce. We compared the resistance to degradation of tannin-protein precipitates from both species, by monitoring C & N dynamics in humus amended with protein, purified tannins or protein-tannin precipitates. We verified the purity of the precipitates using solid-state ^{13}C NMR spectra. We compared the ability of mycorrhizal fungi associated to both species to grow on media amended with tannin-protein complexes as the principal N source.

- The protein precipitation capacity of *Kalmia* tannins was superior to those of black spruce. Humus amended with protein increased both mineral and microbial N, whereas humus amended with tannin-protein precipitates increased dissolved organic N. Mycorrhizal fungi associated with *Kalmia* showed better growth than those associated with black spruce when N was provided as tannin-protein precipitates.

- Our data suggest that *Kalmia* litter increases the amount of soil N sequestered as tannin-protein complexes, which may improve the competitive ability of *Kalmia* relative to black spruce by favouring N uptake by mycorrhizas associated with the former.

Introduction

Most of the nitrogen (N) entering the soil system consists of high-molecular-weight organic substrates such as proteins. Because roots only absorb mineral N (NH_4^+ or NO_3^-) or low-molecular-weight organic N molecules such as amino acids (Nasholm *et al.*, 1998), proteins must first be decomposed and mineralized before plants can have access to soil N. The first stages of protein degradation in soil are catalyzed by exo-enzymes that are released into the soil solution by microorganisms. These enzymes are understood to be inefficient in the presence of condensed tannins (Joanisse *et al.*, 2007), which are plant-produced polymers of three-ring flavanols that form stable cross-links with protein.

The role of plant-produced tannins in controlling soil N cycling has frequently been evoked and studied in boreal forest–ericaceous shrub systems (e.g. Bradley *et al.*, 2000a,b). Lebel *et al.* (in press) showed that the presence of sheep laurel (*Kalmia angustifolia* L.), a common boreal ericaceous shrub, was related to low soil N mineralization rates and stunted growth of regenerating black spruce (*Picea mariana* (Mill.) BSP) seedlings, and they suggested that this resulted from binding of *Kalmia* litter tannins to soil proteins. Joanisse *et al.* (2007) found that *Kalmia* leaves contained five time more condensed tannins than black spruce needles, which further corroborates this premise that *Kalmia* leaf litter may interfere with soil N cycling. However, tannins produced by different plants have different chain lengths and stereochemistries, and these structural differences could logically result in different functional properties (Kraus *et al.*, 2003). With regards to *Kalmia* and black spruce, previous studies have shown definite structural differences in the condensed tannins that they each produce (Nierop *et al.*, 2005; Joanisse *et al.*, 2007), and this could affect their respective N-binding capacities.

The ability of tannins to bind protein should not be confused with the stability of these precipitates to resist degradation in soil. For example, Howard & Howard (1993) found that tannins produced by a range of tree and shrub species sequestered different amounts of protein, and the resulting precipitates differed substantially in their ability to resist degradation. If litter tannins of a given plant species form resistant bonds with soil proteins, then we should expect an increase in dissolved organic N (DON) in soil solution and a concomitant decrease in mineral N (Northup *et al.*, 1995). On the other hand, if tannins and tannin-protein complexes readily decompose, then they effectively would act as energy-yielding substrates to soil microorganisms. In this case, what could be perceived as lower mineral N due to the sequestration of soil protein would actually be the result of greater microbial immobilization of N. Thus attempts to evaluate the stability of tannins and tannin-protein complexes in soil should include measurement of microbial respiration, soil mineral N, DON, and microbial N content.

Many boreal forest plants growing in tannin-rich soil environments may present biological systems that have evolved to circumvent the need for exogenous N mineralization pathways. For example, mycorrhizal symbionts associated to some boreal plants have been shown to produce enzymes that degrade polymeric organic matter and subsequently assimilate the resulting hydrolysates (Read & Perez-Moreno, 2003; Read *et al.*, 2004). The taxonomic diversity of mycorrhizal species being, however, very high – 5000–6000 boreal species according to Buscot *et al.* (2000) – we expect the functional diversity of mycorrhizal species colonizing different plants to be high as well. In order to understand, therefore, the ability of *Kalmia* and black spruce to cope with N sequestration in tannin-rich soil environments, there is a need to evaluate how various species of their respective mycorrhizal symbionts can grow in the presence of tannins or tannin-protein precipitates.

In the present study, our first objective was to compare the protein-precipitation capacities of purified tannins and leaf extracts from *Kalmia* and black spruce. Since the capacity of tannins to precipitate protein is not tantamount to the stability (i.e. resistance to degradation) of the precipitates, our second objective was to compare C and N dynamics in forest floor material amended with tannins and protein-tannin complexes from *Kalmia* and black spruce. In so doing, we verified the purity of tannin-protein precipitates formed from *Kalmia* and black spruce leaf extracts by comparing their solid-state ^{13}C nuclear magnetic resonance (NMR) spectra to those of purified tannins and purified protein. Our third objective was to compare the ability of different mycorrhizal species frequently associated with *Kalmia* or black spruce, to grow on media amended with purified tannins as well as on media containing *Kalmia* or black spruce tannin-protein complexes as the principal N source. Given the established notion that *Kalmia* tannins contribute significantly towards reducing the cycling of soil N, we hypothesized that these would have a greater protein binding capacity and a greater stability in soil than black spruce tannins. We further hypothesized that mycorrhizal fungi associated to black spruce would grow less on media containing tannin-protein complexes as the principal N source than mycorrhizal fungi associated to *Kalmia*.

Material and Methods

Experiment 1: Protein-precipitation capacity of tannins

Two methods were used to compare the protein-precipitation capacities of *Kalmia* and black spruce condensed tannins, and that of hydrolysable tannic acid. Given that the emphasis of our study was on the structural and functional differences between the tannin types, we used commercially-available bovine serum albumin (BSA) as the test protein, as previously used in similar studies (e.g., Bending and Read, 1996; Mutabaruka *et al.*, 2007). We first used the

radial diffusion assay as described by Hagerman (1987). This assay measured the formation of insoluble protein-tannin complexes in agar plates containing 10 ml of 0.1 % BSA solution. Condensed tannins from *Kalmia* leaves and black spruce needles had previously been purified according to the method described by Preston (1999). Solution ^{13}C NMR showed both were predominantly procyanidin units (2 OH groups on the B ring) with C2-C3 *cis* stereochemistry; however, average chain length was 6.0 for black spruce and 2.3 for *Kalmia* (Nierop *et al.*, 2005; Joanisse *et al.*, 2007). Three solution concentrations (12.5, 25 and 50 mg ml^{-1}) of *Kalmia* and black spruce tannins, and generic tannic acid (Sigma-Aldrich), were prepared in 50% acetone:water. For each of the nine treatments (i.e., 3 tannin types x 3 concentrations), four replicate agar plates were used. Four BSA-agar cores (3 mm dia.) were removed from each plate and 20 µl aliquots of tannin solutions were pipetted into the resulting wells. Four control agar plates were cored and amended in a similar manner with solution consisting of 50% acetone:water only. All plates were incubated at 30 °C for 96 h, and the diameter of the opaque precipitation ring around each well was measured along two perpendicular axes. The depth of the agar around each well was determined using callipers in order to estimate the volume and weight of protein that was precipitated around each well.

Precipitation capacities of the three tannin types, and those of *Kalmia* leaf and spruce needle extracts, were also measured in solution. Three grams of each tannin type were dissolved in 500 ml H_2O, and filter-sterilized through glass filter papers of 0.80, 0.45 and 0.22 µm pore-size diameter. Fresh green *Kalmia* leaves and spruce needles were freeze-dried, finely ground with a ball mill, and 50 g were transferred to brown glass bottles to which were added 500 ml of acetone:water (70:30). Bottles were shaken for 1 h and solutions were centrifuged at 3000 rev min^{-1}. Supernatants were filtered (Whatman No.5) whereas pellets were re-suspended in 500 ml of 70% acetone:water and the extraction procedure repeated a second time. Solutions from both extractions were combined and the acetone was removed from solution by roto-evaporation at 30 °C. The remaining aqueous *Kalmia* and black spruce extracts were filter-sterilized and stored in sterilized bottles. The pH of the five solutions were *Kalmia* tannins

=3.99, black spruce tannins = 3.61, tannic acid = 4.00, *Kalmia* extract = 4.02, and black spruce extract = 3.77. A solution containing 2.5 g l^{-1} of BSA in sodium acetate buffer (10 μM, pH 4.5) was prepared, filter sterilized and kept in a sterile bottle at 4 °C. Each tannin solution and each foliar extract were transferred in 2, 5, 10 and 15 ml in duplicate sterile centrifuge tubes, and the volume in each tube was raised to 20 ml using the BSA solution. This resulted in tannin:BSA (w/w) ratios of 0.27, 0.80, 2.4 and 7.2, and leaf:BSA ratios (w/w) of 7.4, 22.2, 66.7 and 200.0. The solution mixture in all 40 tubes was mixed with a vortex and kept at 4 °C for 24 h. These were then centrifuged (4000 rev min^{-1}) and supernatants discarded. Pellets were rinsed with H_2O, vortex-mixed, centrifuged, and supernatants discarded once again. Precipitates were freeze-dried, weighed and analyzed colorimetrically for total N following acid digestion (sulfuric acid – hydrogen peroxide mixture) using a Technicon Autoanalyser (Pulse Instrumentation, Saskatoon, Canada). The amount of BSA precipitated in each solution was calculated using a mass conversion factor of 0.16 total-N:BSA.

Experiment 2: Stability of protein-tannin complexes in soil

Forest floor humus material was collected from a 10-y-old black spruce cutover located near the Town of Senneterre, Québec, Canada (ca. 48° N, 76° W). Mean annual temperature is 0.5 °C and mean annual precipitation is 972 mm. Soils of the region are mainly Humo-Ferric Podzols and the drainage at this particular site is classified as "mesic" (Blouin & Berger, 2001). The site was characterised by an average 10 cm deep organic F-H layer. The groundcover consisted of a feathermoss (*Pleurozium schreberi* (Brid.) Mitt) mat and the shrub layer was dominated by *Kalmia angustifolia*. Samples (ca. 500 g) of organic F-H layer (Soil Classification Working Group, 1998) material were collected every 5 m along two 50 m transects and bulked to create a single sample. The material was sieved to pass a 5 mm mesh, transported on ice to the *Laboratoire d'écologie des sols – Université de Sherbrooke* and stored at 4°C until the experiment began, two weeks later. Five subsamples were finely

ground using a Retsch model MM200 ball mill (Retsch GmbH & Co., Haan Germany), these were acid digested and analyzed colorimetrically for total N and P by the indophenol blue and the vanado-molybdo-phosphoric acid methods respectively. Forest floor pH was measured electrometrically from aqueous suspensions (soil:water = 1:10).

Exactly 10 g (3.75 g dry wt equiv.) of fresh humus material were transferred into 108 plastic jars (500 ml) and left five days to condition at room temperature. Each jar was assigned one of nine treatments, which were replicated 12 times. These comprised eight amendments (BSA, *Kalmia* and black spruce tannins, *Kalmia* and black spruce tannin-BSA precipitates, *Kalmia* and black spruce extract-BSA precipitates, and an unamended control). Tannin-protein complexes were prepared as described above by mixing 10 ml of each tannin solution or leaf extract (described above) and 30 ml of BSA solution. This resulted in tannin:BSA and leaf:BSA ratios of 0.80 and 22.2 respectively, thereby precipitating about 95% of the BSA from all solutions. In order to ensure a uniform distribution in the humus, substrates were applied with talc in 500 mg mixtures. We used 30 mg tannin-protein complexes, 20 mg of purified tannins and BSA, and 500 mg talc for the control. The C and N concentration of each amendment was determined using a Vario Macro CN Analyzer (Elementar GmbH, Germany). In order to monitor CO_2 evolution, a glass vial (50 ml) was fixed with adhesive tape onto the interior face of each jar to which we added 20 ml of 0.5 M KOH solution. Jars were immediately sealed with air tight lids. Empty jars were also prepared to correct for ambient CO_2 concentrations.

Three jars of each treatment were destructively sampled 15 min after amendments were applied (t=0), as well as after 2, 7 and 28 days. Upon opening each jar, 20 ml of 1 N $BaCl_2$ solution were added to bottles containing KOH solution, and these were titrated with 0.1 N HCl using a 0.05% thymolphthalein-ethanol indicator. HCl volumes were converted to mg CO_2-C.

Soil mineral N concentration in each jar was determined by extracting 4.5 g (fresh wt) soil subsamples with 40 ml of 0.5 M K_2SO_4 solution. After shaking for 1 h, extracts were filtered (Whatman No.5) and analyzed colorimetrically for NH_4^+-N (nitroprusside–hypochlorite–salycilate) and NO_3^--N (Cd reduction–sulphanilamide) concentrations (Mulvaney, 1996). NO_3^--N concentrations were consistently below the detection limit of our instrument (0.06 μg ml^{-1}). The remaining extract was subsequently filtered through a 0.45 μm syringe filter and analysed for total dissolved N (TDN) by persulphate oxidation (Cabrera & Beare, 1993). Briefly, 10 ml of persulphate solution was added to duplicate 5 ml subsamples of the filtered extracts, the mixtures were autoclaved at 121°C for 45 min and then analysed for NO_3^--N. Dissolved organic N (DON) concentration was calculated by subtracting NH_4^+-N from TDN concentrations.

Microbial biomass N (N_{mic}) in each jar was determined by the chloroform fumigation extraction technique (Brookes *et al.*, 1985). A 4.5 g (fresh wt) subsample of soil from each jar was transferred to a 125 ml Mason jar and placed inside a desiccator. A glass dish containing 50 ml of distilled $CHCl_3$ and boiling chips was placed on the bottom of the desiccator, and humus subsamples were fumigated under vacuum for 24 h. Fumigated samples were then extracted in 0.5 M K_2SO_4 and total dissolved nitrogen (TDN) concentration was measured (as described above). To calculate N_{mic}, TDN of the non-fumigated samples (described above) were subtracted from those of fumigated samples.

The chemical nature of leaf extract precipitates was verified by comparing their solid-state ^{13}C NMR spectra with cross-polarization and magic-angle spinning (CPMAS NMR), to those of BSA, purified tannins and tannin-BSA precipitates, using a Bruker MSL 300 spectrometer (Bruker Instruments, Karlsruhe, Germany) operating at 75.47 MHz (Lorenz *et al.*, 2000; Preston & Trofymow, 2000). Dry samples were spun at 4.7 kHz in 7 mm dia zirconium oxide rotors. Spectra were acquired with a 1 ms contact time, 2 s recycle time and 4000-8000 scans,

and were processed using 30 Hz line-broadening and baseline corrections in Win-NMR 6.0 (Bruker Instrument Inc., Germany). Peak heights of pure tannins and BSA were adjusted to peaks heights of leaf extract precipitates. In order to verify the purity of tannin-protein precipitates formed from *Kalmia* and black spruce leaf extracts, the corrected BSA spectrum was plotted against the spectra of both tannin types and both tannin-BSA precipitates, and the latter were plotted against the spectra of leaf extract precipitates.

Experiment 3: Mycorrhizal fungus growth in the presence of tannins and tannin-protein complexes

Four mycorrhizal fungi commonly found in black spruce forests or on ericaceous shrubs, were purchased from the University of Alberta Microfungus Collection (Edmonton, Canada). Two species, *Hebeloma crustuliniforme* (UAMH 5460) and *Cenococcum geophilum* (UAMH 6145), are ectomycorrhizal (ECM) endophytes common to black spruce (Byrd *et al.*, 2000; Kranabetter *et al.*, 2005; Robertson *et al.*, 2006). Both species can grow on organic N sources (Abuzinadah and Read 1986; Zhu *et al.*, 1990), phenolic compounds and/or leaf extracts (Mallik & Zhu, 1995; Mallik & Zhu, 1998). The third species was *Rhizoscyphus ericae* (UAMH 8680, formally named *Hymenocyphus ericae*), an ericoid mycorrhiza (ERM) common among ericaceous shrubs (Massicotte *et al.*, 2005) that can grow on organic N sources and phenolic compounds (Bending & Read, 1996,1997; Read & Perez-Moreno, 2003; Read *et al.*, 2004). The fourth species was the dark septa mycorrhizal (DSM) endophyte *Phialocephala fortinii* (UAMH 7137), which is commonly associated with roots of both ericaceous shrubs (Massicotte *et al.*, 2005) and black spruce (Yamasaki *et al.*, 1998; Massicotte *et al.*, 2005; Olsrud *et al.*, 2007), but may actually be pathogenic to conifers (Richard & Fortin, 1974; Wilcox & Wang, 1987). *P. fortinii* can also grow on a wide range of organic compounds (Caldwell *et al.*, 2000) including *Kalmia* leaf extracts (Titus *et al.*, 1995).

The experiment consisted of growing the four mycorrhizal species on sterilized Modified Melin Norkrans (MMN) nutrient medium without malt extract in Petri dishes solidified with agar, either in the presence of purified *Kalmia* or black spruce tannins, tannic acid, BSA, or tannin-BSA precipitates prepared as in experiment #2. A non-amended control (MMN only) was included as an eighth treatment. For the three tannin treatments, we dissolved 450 mg of each tannin type in 75 ml of H_2O, solutions were filter sterilized (0.22 μm) and mixed with the autoclaved MMN media in a proportion of 1:20 (v/v). For the BSA treatment, we dissolved 1.0 g BSA in 250 ml H_2O, filter sterilized (0.22 μm) with a low protein binding filter paper and mixed the solution with the autoclaved MMN medium in a proportion of 1:20 (v/v). For the three tannin-protein precipitate treatments, we added 500 mg of each precipitate per litre of autoclaved MMN medium. Mineral N was added (250 mg $NH_4H_2PO_4$ l^{-1} solution) to the purified tannin and control treatment solutions, whereas glucose was added to all eight solutions (5.0 g l^{-1}). Given that boreal forest soils are typically acidic, the pH of each solution was adjusted to 4.8, the lowest pH at which agar can solidify (Marx, 1969). We poured 25 ml of each agar growth medium into twenty 9 cm dia. Petri dishes. The amount of total N added to each Petri dish was: Control = 761, *Kalmia* tannins = 761, black spruce tannins = 761, tannic acid = 761, BSA = 795, *Kalmia* extract-BSA = 1000, black spruce extract-BSA = 988, and tannic acid-BSA = 1217 μg N per Petri dish. Five Petri dishes of each growth medium were inoculated with a single agar plug (4.0 mm dia.) taken from colonies of each mycorrhizal species growing on MMN agar medium. The 160 Petri dishes were sealed with parafilm, and incubated for 60 days in the dark at 20°C. The Petri dishes were then set in a thin layer of hot (50-60 °C) water in order to melt the agar, the mycelia were collected on pre-weighed glass filter papers and rinsed twice with distilled water. Filter papers and mycelia were freeze-dried and weighed.

Statistical analyses

In experiment 1, simple linear regressions were performed to test the relationships between tannin concentration and the amount of BSA that was precipitated in the radial diffusion assay, and analysis of covariance was used to compare the slopes of these regression lines. Two-way ANOVAs were used to test the effects of tannin type, tannin concentration (or ratios to BSA), and the interaction between tannin type x tannin concentration on the N content of precipitates and on the amount of BSA precipitated from solution. When significant interactions were found, one-way ANOVAs within each tannin type were performed. Ryan-Einot-Gabriel-Welsch F (REGW-F) post-hoc tests were used to detect significantly different means. Data in experiment 2 were analyzed in the same way as in experiment 1, with incubation time and humus amendments as the experimental factors. For data in experiment 3, one-way ANOVAs were used to test the effects of the eight growth media on mycelial mass of each mycorrhizal species. We then performed one-way ANOVAs to compare mycelial mass of each mycorrhizal species within each N source (i.e. NH_4^+ and BSA). One-way ANOVAs and single degree of freedom orthogonal contrasts were also performed to compare relative mycelial mass in the tannin-protein media relative to the mycelial mass in the NH_4^+ and BSA media between mycorrhizal species. Statistical analyses were performed using SPSS 11.01 (SPSS Inc., Chicago, IL, USA) software. Before each analysis, we verified that the data conformed to assumptions of normality and homogeneity of variance, and used ln-transformations when necessary to meet these assumptions. The level of significance for all tests was set to $P \leq 0.05$.

Results

Experiment 1: Protein precipitation capacity of tannins

Two-way ANOVA revealed significant ($P<0.001$) effects of tannin type, tannin concentration, and their interaction term, on the amount of BSA precipitated in the radial diffusion assay. At all three tannin concentrations, tannic acid precipitated significantly more BSA than *Kalmia* and black spruce tannins (Fig. 1). For all three tannin types, the amount of precipitate increased linearly over the range of concentrations that were used and analysis of covariance revealed a significantly ($F_{2,31}=78.6$, $P<0.001$) higher regression slope for tannic acid than for *Kalmia* and black spruce tannins (Fig. 1).

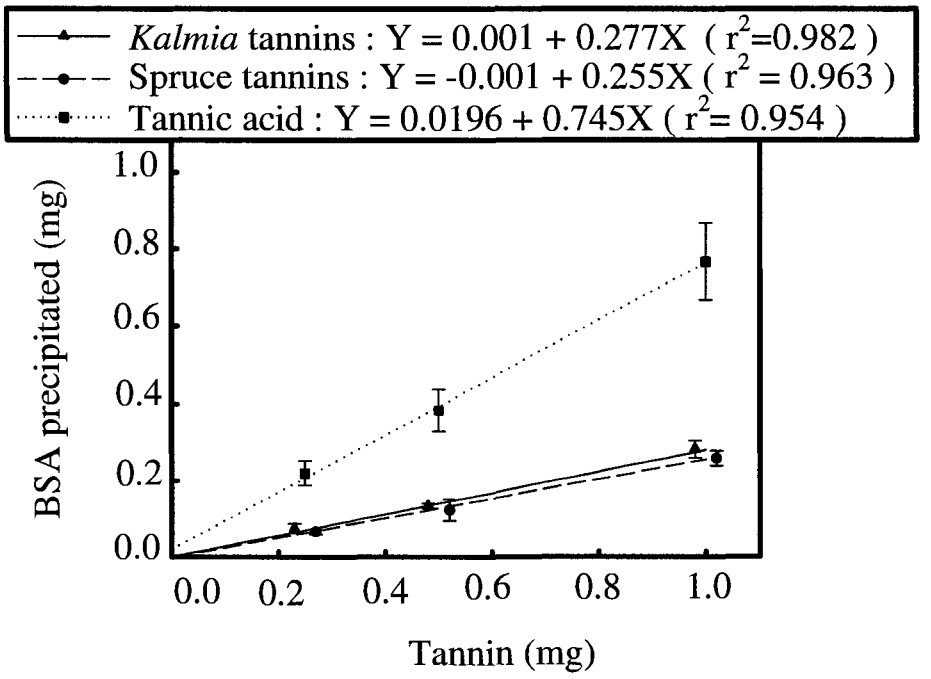

Figure 1. Effect of tannin concentrations on the amount of bovine serum albumin (BSA) precipitated during radial diffusion assays. All regressions are significant at $P<0.0001$. Each point represents the mean value of 4 Petri dishes; vertical lines = 1 S.D. Points depicting *Kalmia* and spruce tannins have been jittered along the X-axis for clarity.

Two-way ANOVAs revealed significant ($P<0.001$) effects of tannin type, tannin:BSA ratios, and their interaction term, on the N concentration of precipitates and on the amount of BSA precipitated (Fig. 2a,b). For each tannin type, the N concentration of precipitates decreased

with an increasing ratio of tannin:BSA (Fig. 2a). One-way ANOVAs and post-hoc REGW-F tests revealed significantly (P=0.014) higher N concentrations for *Kalmia* and black spruce tannins than for tannic acid at the 0.8 tannin:BSA ratio, and a significantly (P<0.022) higher N concentrations for *Kalmia* tannins than for black spruce tannins and tannic acid at 2.4 and 7.2 tannin:BSA ratios (Fig. 2a). On the other hand, there was significantly (P=0.001) more BSA precipitated with tannic acid than with *Kalmia* or black spruce tannins at the lowest (0.27) tannin:BSA ratio (Fig. 2b). At higher tannin:BSA ratios, most of the BSA had precipitated.

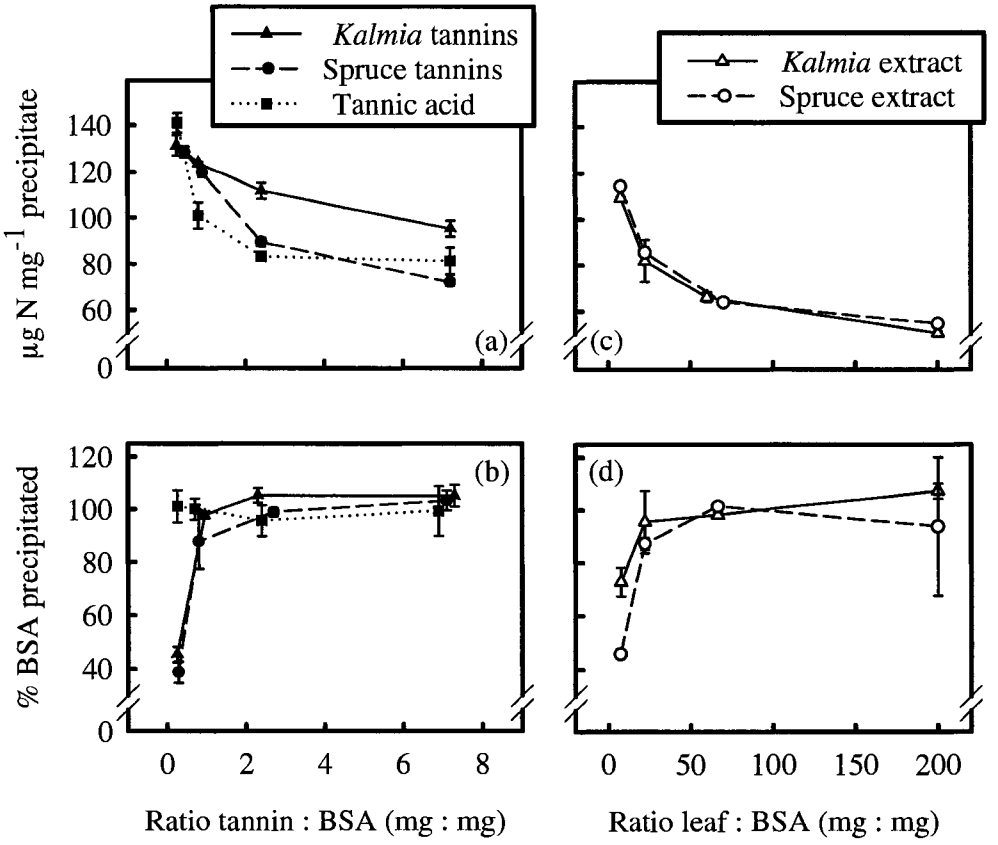

Figure 2. Effects of increasing ratios of tannins to BSA (a,b), and leaf extract to BSA (c,d), on the percentage of total BSA precipitated from solution and on the nitrogen concentration of these precipitates. Each point represents the mean of duplicate solutions; vertical lines = 1 S.D. Overlapping points have been jittered along the X-axis for clarity.

For leaf extracts, two-way ANOVA revealed significant effects ($P<0.001$) of leaf:BSA ratios on the N concentration of precipitates (Fig. 2c). These decreased with an increasing ratio of leaf:BSA. Two-way ANOVA revealed significant effects of leaf type ($P=0.018$) and leaf:BSA ratio ($P<0.001$) on the amount of BSA precipitated from solution (Fig. 2d). More specifically, *Kalmia* extracts precipitated more BSA from solution than black spruce extracts at the lowest (7.40) leaf:BSA ratio (Fig 2d). At higher leaf:BSA ratios, the proportion of BSA that was precipitated varied between 91–100%.

Table 1. C and N contents of the 500 mg talc-substrate mixtures added to 3.75 g (dry wt equiv) humus samples in Experiment 2.

	Control	BSA	KT	KT-BSA	KE-BSA	BST	BST-BSA	BSE-BSA	TA-BSA
N (mg)	bdl	3.01	bdl	2.67	2.42	bdl	3.24	2.37	2.92
C (mg)	bdl	10.05	11.12	12.25	17.60	12.20	15.68	16.75	16.02

Note: Control = talc only; BSA = bovine serum albumin; KT and BST are *Kalmia* and black spruce purified tannins; KE and BSE are *Kalmia* and black spruce leaf extracts; TA = tannic acid; bdl = below detection limit.

Experiment 2: Stability of tannin-protein complexes in soil

Initial characteristics of the humus used for this experiment were as follows: total N = 13.73 mg g^{-1}; total P = 0.56 mg g^{-1}; organic matter = 89 %, pH = 3.6. The C and N contents of each amendment are given in Table 1. NMR spectra of *Kalmia* extracts-BSA, BSA and *Kalmia* tannins are shown in Figure 3a and those of black spruce extracts-BSA, BSA and black spruce tannins are shown in Figure 3b. Tannin and BSA spectra were consistent with previous spectra and chemical-shift data (Preston *et al.*, 2000). All peaks of the plant extract-

BSA spectra were found in the combined spectra of BSA and purified tannins, indicating that leaf extract precipitates are primarily composed of pure tannins and BSA. This was confirmed by adding the spectra of BSA to those of purified tannins, which resulted in spectra that are nearly identical to those of leaf extract precipitates (Fig. 3c,d).

Two-way ANOVA revealed significant interactions between incubation time and humus amendments in controlling cumulative CO_2-C respired. Subsequent one-way ANOVAs revealed that the effect of amendments on cumulative CO_2-C was only significant after 28 days incubation. On this date, post-hoc REGW-F analysis revealed significantly lower values in the black spruce tannins than in the BSA treatment (Fig. 4a).

Two-way ANOVA revealed significant interactions between incubation time and humus amendments in controlling NH_4^+-N concentrations. Subsequent one-way ANOVAs revealed a significant effect of amendments after 2, 7 and 28 days incubation. At day 2, post-hoc REGW-F analyses revealed significantly ($P<0.01$) higher NH_4^+-N concentrations in the control and BSA than in the other treatments (data not shown). At day 7 (data not shown) and day 28 (Fig. 4b), BSA amended humus had significantly ($P<0.01$) higher extractible NH_4^+-N concentrations than all other treatments. Assuming that this net increase in NH_4^+-N over the control treatment was attributable to the mineralization of BSA, and that no losses of N through volatilization occurred, results suggest that 32% of BSA-nitrogen was mineralized after 28 days incubation.

Two-way ANOVA revealed significant interactions between incubation time and humus amendments in controlling DON. Subsequent one-way ANOVAs revealed a significant effect of amendments after 0, 2 and 28 days incubation. At day 0 and day 2, post-hoc REGW-F analyses revealed significantly ($P=0.04$ for both) lower DON concentrations in the control

compared to other treatments (data not shown). At day 28, DON concentrations were significantly (P=0.04) lower in the BSA than in the other treatments (Fig. 4c).

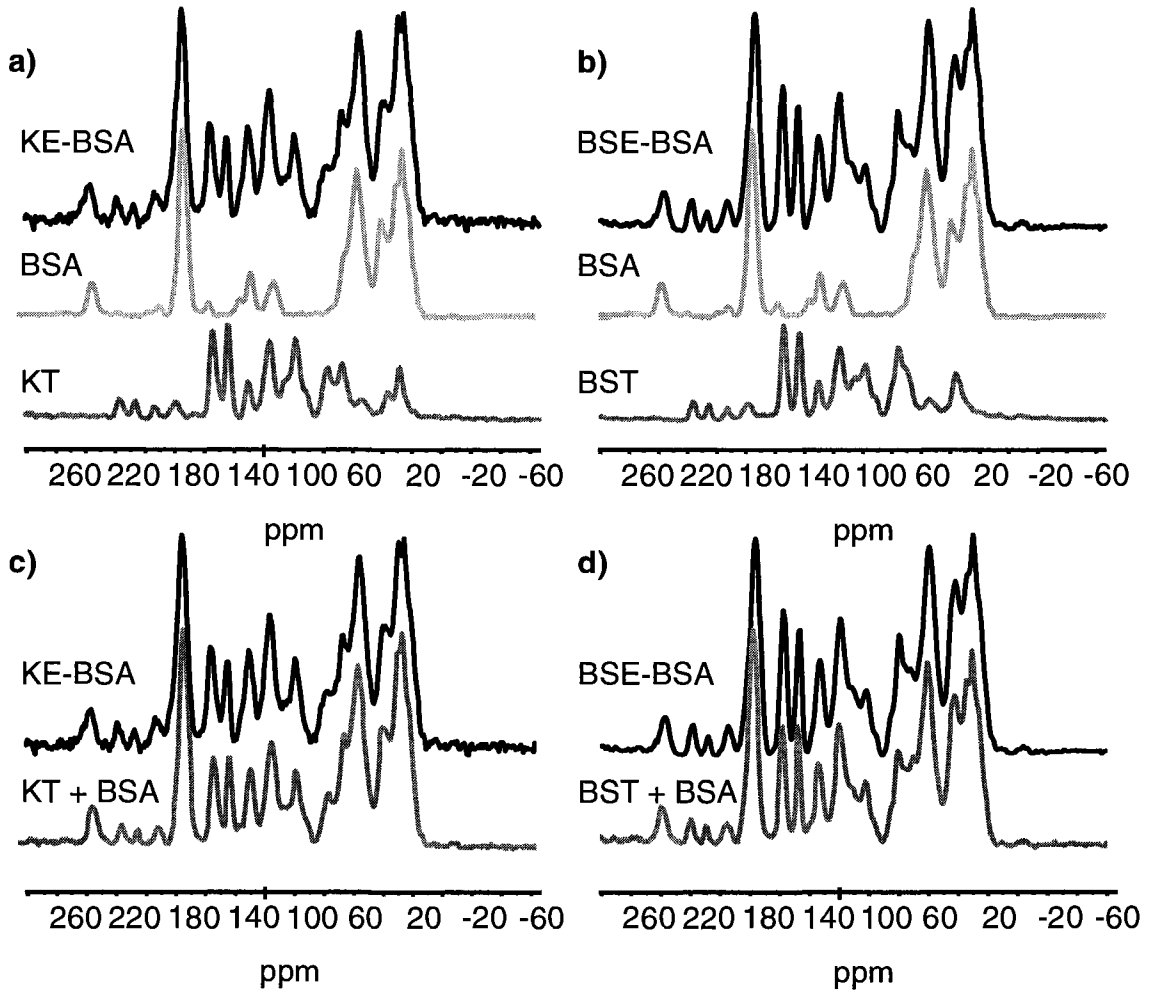

Figure 3. ^{13}C CPMAS NMR spectra of (a, b) Kalmia and black spruce leaf extract-BSA precipitates (KE-BSA and BSE-BSA respectively), of BSA, and of purified Kalmia and black spruce condensed tannins (KT and BST respectively). Lower frames (c, d) compare the spectra of leaf extract-BSA precipitates with the summed spectra of BSA and purified tannins.

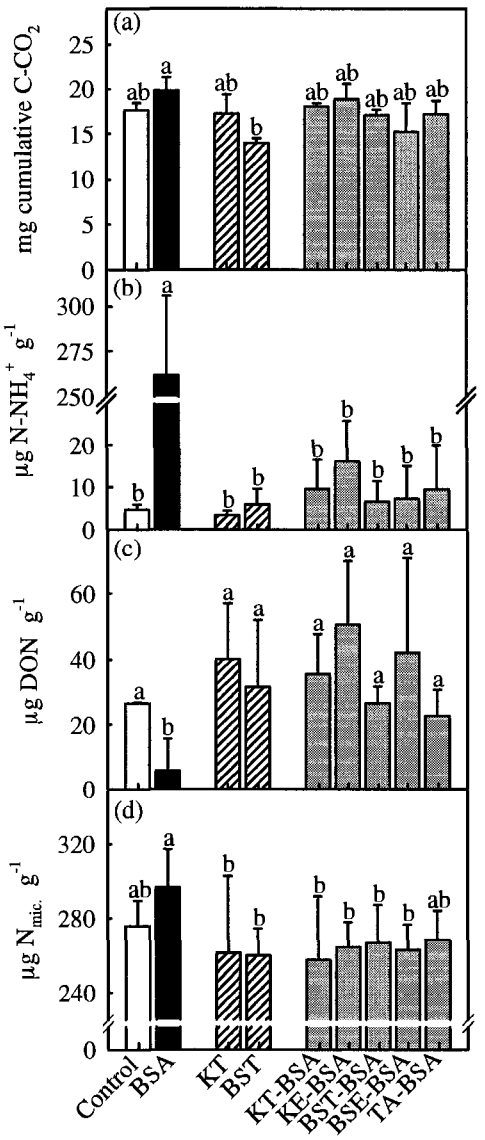

Figure 4. Effects of BSA (black), condensed tannins (cross-hatch) and tannin-BSA precipitates (grey) on (a) cumulative respiration, (b) mineral N, and (c) dissolved organic N after 28 days incubation, as well as on microbial N pooled across dates. Substrate abbreviations are: Control = talc only; BSA = bovine serum albumin; KT and BST = Kalmia and black spruce tannins; KE and BSE = Kalmia and black spruce leaf extracts; TA = tannic acid. Bars represent means of three (a,b,c) or twelve (d) experimental units; vertical lines = 1 S.D. Different lower-case letters within each frame indicate significantly different means according to REGW-F tests.

Two-way ANOVA revealed a significant effect of humus amendments in controlling N_{mic}. Post-hoc REGW-F analysis of the data pooled across dates revealed significantly (P=0.006) higher N_{mic} concentrations in the BSA than in the other amended treatments (Fig. 4d).

Experiment 3: Mycorrhizal growth in the presence of tannin-protein complexes

One-way ANOVAs revealed significant effects of growth media on mycelial mass of all mycorrhizal species except *P. fortinii* (Fig. 5). For the other three species, mycelial mass was lower in growth media containing tannin-protein precipitates than those with NH_4^+ as the N source (Fig. 5a,b,c). Within the four media with NH_4^+ as the N source, there was significantly higher mycelial mass in the *Kalmia* tannins than in the control treatments for both *H. crustuliniforme* (P=0.04) and *C. geophilum* (P=0.032) (Fig. 5a,b), and higher mycelial mass in the tannic acid than in the control treatment (P=0.028) for *R. ericae* (Fig. 5c). Within the four treatments with BSA as the N source, mycelial mass was significantly (P<0.002) higher with BSA alone than with each tannin-BSA precipitates for species *H.crustuliniforme* (Fig. 5a), *C. geophilum* (Fig. 5b) and *R. ericae* (Fig. 5c). Compared to the other three mycorrhizal species, mycelial mass of *C. geophilum* (Fig. 5b) growing on media with BSA alone was low relative to the four media with NH_4^+ as the N source. Single degree of freedom orthogonal contrasts revealed that, relative to the control media, mycelial masses of the two ECM species (*H. crustuliniforme* and *C. geophilum*) growing on the three tannin-BSA media were significantly lower (P<0.006) than those of *R. ericae* and *P. fortinii*.

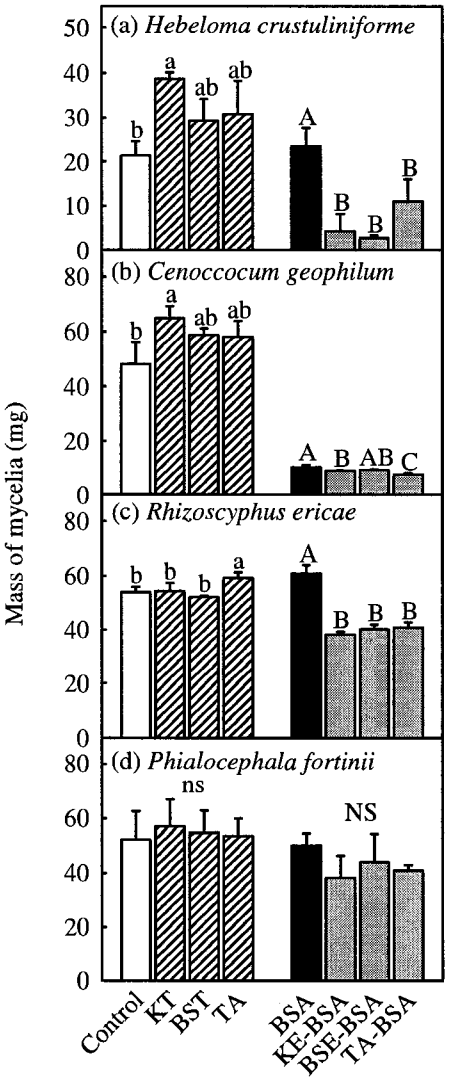

Figure 5. Mycelial mass of a) *Hebeloma crustuliniforme*, b) *Cenococcum geophilum*, c) *Rhizoscyphus ericae*, and d) *Phialocephala fortinii* grown 60 days on various substrates whose abbreviations are: Control = MMN nutrient medium; (KT, BST and TA) = (*Kalmia* tannin, black spruce tannin, and tannic acid) + NH_4^+; BSA = bovine serum albumin; (KE-BSA, BSE-BSA and TA-BSA) = (*Kalmia* leaf extracts, black spruce needle extracts, and tannic acid) + BSA. Different lower case letters within each frame indicate significant differences (REGW-F test) between control and purified tannin media, all containing mineral N, whereas different upper case letters indicate significant differences between protein-tannin complexes and BSA media, all containing organic N. Vertical bars = 1 S.D.

Discussion

Results of the radial diffusion assay (Fig. 1) suggest that *Kalmia* tannins do not precipitate more protein than those produced by black spruce. While this assay is rapid and inexpensive, and has thus been extensively used in the past, caution is warranted in interpreting the results. Firstly, protein binding capacity is inferred from the volume of the opaque precipitation ring around each well in the agar plates. The data follow, therefore, a binary scale (opaque vs. translucent) that assumes, perhaps falsely, the uniform precipitation of protein within the opaque rings for all tannin types. Secondly, the volume of the opaque ring can be related to the ability of tannins to diffuse through the agar medium as it is a measure of the protein binding capacity. In this respect, Joanisse *et al.* (2007) found that *Kalmia* and black spruce tannins consisted of polymers with average chain lengths of 2.3 and 6.0 three-ring flavanol units respectively, which would lead us to expect slower diffusion of black spruce tannins due to the higher molecular weight. However, the low apparent chain length of *Kalmia* root and shoot tannins (Nierop *et al.*, 2005; Joanisse *et al.*, 2007) may conceal other structural features such as lateral branching, or incorporation of *p*-coumaric acid (Nierop *et al.*, 2005), which may also impede its diffusion. Based on the volume of the opaque rings, our results suggest that tannic acid precipitates significantly more protein than condensed litter tannins, which is consistent with other studies (Giner-chavez *et al.*, 1997; Kraus *et al.*, 2003). Again, caution is warranted in interpreting this result, as tannic acid may diffuse more easily through agar medium than condensed tannins. Thus, the caveats associated with the radial diffusion assay warranted the second part of this first experiment, which measured the actual weight and chemical characteristics of tannin-protein precipitates formed in solution, as they would naturally be formed in the soil.

All three tannin types were efficient in precipitating BSA from solution (Fig. 2a,b). Although BSA is not a soil protein, these results could nevertheless be ecologically relevant because

the tannin:BSA ratios that we used corresponded to tannin:N ratios that were either smaller or within the same order of magnitude (i.e. 1.7–45.3) as tannin:N ratios found in *Kalmia* leaves (32.5) and black spruce needles (23.6) (Joanisse *et al.*, 2007). In fact, most of the BSA had precipitated from solution at a tannin:BSA ratio of 0.8, corresponding to a tannin:N ratio of 5.0. Given that the N concentration of precipitates decreased asymptotically with increasing tannin concentration, we posit that there is a "luxury amount" of tannins that can be bound within precipitates as the free protein pool in solution becomes limited. Whether the extra tannins that are bound at elevated tannin:BSA ratios confer greater resistance to the precipitates remains to be determined.

At the lowest tannin:BSA ratio (i.e. 0.27), we found tannic acid to bind more efficiently to BSA in solution than *Kalmia* or spruce tannins. This confirms that the larger opaque rings measured around wells inoculated with tannic acid in the radial diffusion assay were not solely the result of hydrolyzable tannins diffusing faster through agar medium than condensed tannins. The comparison with hydrolysable tannins is, however, of lesser importance given that *Kalmia* and black spruce both lack hydrolyzable tannins in their tissues (Nierop *et al.*, 2005). By far, the salient finding of this experiment is that the amount of protein precipitated per unit mass of tannins is greater for *Kalmia* than for black spruce, an observation that was not apparent with the radial diffusion assay. Thus, if the formation of tannin-protein precipitates in soil is a mechanism by which *Kalmia* can gain a competitive advantage over black spruce, then this strategy is not only ascribed to the higher tannin concentrations found in *Kalmia* litter (Joanisse *et al.*, 2007), but also to the more efficient protein binding capacity of *Kalmia* tannins.

Results of ^{13}C CPMAS NMR analyses (Fig. 3) leave little doubt as to the purity of tannin:protein precipitates formed from leaf extracts and BSA. It is not surprising, therefore, that the N concentrations of precipitates formed from leaf extracts, and the corresponding

amounts of BSA precipitated from solution, responded to variations in leaf:BSA ratios (Fig. 2c,d) exactly as they did to variations in tannin:BSA ratios (Fig. 2a,b). Precipitates formed from leaf extracts and those formed from purified tannins appeared to differ, however, in terms of the species differences that were observed. Here, we noted two inconsistencies: (1) precipitates formed with extracts of both species had similar N concentrations (Fig. 2c), and (2) *Kalmia* leaf extracts precipitated more BSA at the lower leaf:BSA ratio than spruce needle extracts (Fig.2d). It should be noted, however, that tannin concentrations in *Kalmia* leaves are up to five times higher than in black spruce needles (Joanisse *et al.*, 2007), thus any given leaf:BSA ratio would yield a higher tannin:BSA ratio for *Kalmia* extracts, which could reduce the N concentration of precipitates (as per Fig. 2a) to similar levels as those formed from spruce needle extracts, and result in a higher % BSA precipitated. We did not observe significantly more BSA precipitated with *Kalmia* extracts at higher leaf:BSA ratios because extracts from both species precipitated most of the BSA at these concentrations. Hence, data shown in Fig. 2a,b are not altogether inconsistent with those shown in Fig. 2c,d.

Taken collectively, the respirometry data provide evidence that neither *Kalmia* and black spruce condensed tannins, nor precipitates formed from purified tannins and leaf extracts, are energy-yielding substrates to soil micro-organisms. Thus, the substantially lower mineral N concentrations found in tannin-amended and precipitate-amended units, compared to the BSA-amended treatment (Fig. 4b), is not the result of higher microbial immobilization in the former, but of higher N mineralization in the latter. This is further corroborated by observations that microbial N content did not increase in units amended with tannins or precipitates, as they did in those amended with BSA (Fig. 4d).

As we expected, the addition of tannins and precipitates did translate into significant increases in DON early on during the incubation (data not shown), and DON concentrations in these treatments were still generally higher than the control treatment after 28 days (Fig.

4c). DON concentrations after 0 and 2 days incubation were also higher in BSA-amended humus as a result of non-degraded BSA being extracted as DON. The fact that DON in BSA-amended humus was lower than the control treatment after 28 days incubation implies that the degradation and uptake of BSA resulted in a concomitant degradation of native DON. This phenomenon, referred to as co-metabolic mineralization (Horvath, 1972; Criddle, 1993), is one in which soil microbes metabolize certain substrates more efficiently in the presence of certain other substrates. Here, the BSA was a readily-decomposable substrate that provided energy to soil microbes, and this may have stimulated the synthesis of catabolic enzymes in the same way as root-derived carbon in the rhizosphere increases the degradation of more recalcitrant native soil organic matter (Bradley & Fyles, 1995; Allison & Vitousek, 2005).

The addition of pure tannins and NH_4^+ as the sole N source did not impede the growth of any of the tested mycorrhizal species, and in some cases growth was actually improved by the presence of tannins. The fact that *C. geophilum* could not metabolize BSA suggests that some ECM species associated to black spruce do not produce protease enzymes, although the extent of this phenomenon and whether it also occurs with some ericoid mycorrhizal species remains unknown. To our knowledge, our third experiment is the first of its kind to have tested the growth of mycorrhizal species using, as sole N source, precipitates formed from condensed tannins extracted from specific plants, rather than precipitates formed from commercially available hydrolysable tannins (e.g. Bending & Read, 1996; Wu *et al.*, 2005). The most meaningful observation in this experiment is that both ECM species associated with black spruce grew very inefficiently when N was provided in the form of tannin:protein precipitates, whereas both mycorrhizal species associated with *Kalmia* showed better growth on these three substrates.

In summary, our data suggest that the presence of *Kalmia* in boreal black spruce forests increases the amount of soil N sequestered as tannin-protein complexes and, although we

have no evidence that these complexes are more resistant to degradation than those produced with spruce tannins, their accumulation in soil may improve the competitive ability of *Kalmia* relative to black spruce by favouring N uptake by mycorrhizas associated with the former. Future research should attempt to confirm this by investigating N uptake by mycorrhizal and non-mycorrhizal roots of both plant species growing on various tannin-protein complexes.

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CHAPITRE II

EST-CE QUE DES COMMUNAUTÉS DE PLANTES DE FIN DE SUCCESSION SUR DES SOLS ACIDES AUGMENTENT LE RATIO DON :DIN ?

Référence: Joanisse, G.D., Bradley, R.L. et Preston, C.M. Do late-successional tannin-rich plant communities occurring on highly acidic soils increase the DON:DIN ratio ? (Biol. Fert. Soils, sous-presse)

La prédominance des formes d'azote dans le sol change au cours de la succession et selon la dominance de certains types d'espèces végétales. Selon les hypothèses proposées, (i.e., Northup et al., 1995), les plantes de fin de succession de milieux infertiles produisent des litières riches en composés phénoliques qui ralentissent la décomposition et séquestre l'azote sous des formes organiques. De ce fait, suivant la dominance d'espèces de plantes contenant de fortes concentrations en composés phénoliques, le principal pool d'azote en solution se trouve sous la forme organique (DON) et s'ensuit une diminution d'azote sous forme minérale (DIN), augmentant ainsi le ratio DON : DIN. Cette hypothèse a été étudiée dans le contexte de l'envahissement de parterres de coupe par le *Kalmia*, puisque celui-ci contient de fortes concentrations de composés phénoliques dans sa litière. Pour ce faire, nous avons réalisé une expérience dans laquelle nous avons ajouté différentes proportions de feuilles sénescentes de *Kalmia* et d'aiguilles sénescentes d'épinette noire dans du sol forestier provenant de parterres de coupe pour simuler l'envahissement progressif du *Kalmia*. Nous avons ensuite mesuré, tout au long d'une incubation de 46 semaines, les formes d'azotes et leurs ratios, l'activité microbienne ainsi que les tanins condensés. Le ratio DON : DIN ne s'est pas avéré comme un bon indice de la séquestration, puisqu'il a augmenté avec la proportion de *Kalmia* seulement pour une date d'incubation. Cependant, les résultats sont

consistants avec la formation de complexes protéines-tanins, puisqu'il n'y aucune différence de minéralisation de l'azote entre les traitements, et aucune immobilisation microbienne de l'azote et du carbone de litière de *Kalmia*, qui contenait deux fois plus d'azote que la litière d'épinette. Il resterait à revoir la notion de ratio DON : DIN comme indicateur de succession dans des milieux infertiles boréales, et déterminer qu'est-ce qui contrôle ce ratio.

J'ai élaboré et réalisé la plupart des expériences de laboratoire, et ma participation à la rédaction du manuscrit fut importante. Les stagiaires P. Lebel et J. Lafond m'ont aidé pour certaines manipulations de laboratoire et de terrains respectivement. La rédaction du manuscrit a été réalisée avec la grande collaboration de mon directeur, Dr. Robert Bradley ainsi que de la Dr. Caroline Preston qui a donné ces commentaires et qui a révisé le manuscrit.

Abstract

Previous studies suggested that late-successional tannin-rich plant communities increase the amount of dissolved organic N (DON) relative to dissolved inorganic N (DIN) in decomposing litter. We devised an experiment to test this hypothesis by adding varying proportions of black spruce (*Picea mariana*) and tannin-rich *Kalmia angustifolia* litter to forest floor samples collected on six black spruce cutovers. An increasing proportion of *Kalmia* litter increased condensed tannin and total phenolic concentrations over the course of a 46 week incubation. Mineral N concentrations did not vary among treatments in spite of much higher total N concentrations in *Kalmia* litter. This was more likely due to the formation of protein-tannin complexes rather than microbial immobilization of N, as indicated by the decline in available C with increasing *Kalmia* litter on two of the five sampling dates. There was a significant positive linear trend between the proportion of *Kalmia* litter and the DON:DIN ratio on one sampling date (week 13) only. Results suggest that the DON:DIN ratio is controlled by confounding factors (e.g., tannins bonding with non-extractable humus particles) and has limited value for describing ecological succession.

Introduction

Condensed tannins are plant-produced polymers of three-ring flavanols that may be transferred to the soil via plant litter (Kraus et al. 2003). Subsequently, tannins may affect soil N cycling, notably by precipitating soil and litter proteins and retarding N mineralization (e.g. Handley 1961). Following this idea, Northup et al. (1995) showed a positive relationship between tannin concentrations in decomposing *Pinus muricata* D. Dons litter and the amount of dissolved organic N (DON) relative to dissolved inorganic N (DIN). They suggested that the DON:DIN ratio reflected the "convergent evolution of tannin-rich plant communities on highly acidic and infertile soils throughout the world". Studies measuring DON:DIN release in soils amended with different litter types are, however, scarce and those that have attempted to measure this ratio following the addition of purified tannins to soil have often failed to confirm that such a relationship exists (Holub and Lajtha 2004; Kanerva et al. 2006). More data are required to validate Northup's proposition, which nevertheless offers a sensible framework for understanding the ecological consequences of elevated tannin concentrations on ecosystem processes.

Here, we devised an experiment based on black spruce *(Picea mariana (Mill.) BSP) – Kalmia angustofolia* L. plant communities, for which the effects of litter tannins in controlling successional pathways have regularly been evoked (Mallik 2003). *Kalmia* is an ericaceous shrub that establishes itself through rhizomatous growth in the subcanopy of boreal black spruce forests, and becomes the dominant late-seral species following stand disturbance. The proliferation of *Kalmia* is often accompanied by a slow down of N cycling in the forest floor, and this has been attributed to the high tannin concentrations found in *Kalmia* leaf litter (Inderjit and Mallik 1996; Bradley et al. 2000; Nierop et al. 2006), which can be five times higher than in black spruce needle litter (Joanisse et al. 2007). Hence, the ecology of the black spruce – *Kalmia* successional pathway is comparable to that of the pygmy pine forests studied by Northup et al. (1995). The aim of our study was to perform an experiment, similar

to the one performed by Northup et al. (1995), that would simulate changes in leaf litter mixtures that could occur during the progressive invasion of *Kalmia* on black spruce cutovers. We tested whether an increase in *Kalmia* litter resulted in higher DON:DIN ratios and, if so, whether this agreed with the paradigm proposed by Northup et al. (1995), or whether this resulted from higher immobilization of mineral N that could be inferred by higher soil available C.

Material and methods

Numerous (~25) forest floor F-layer humus samples were collected on each of six black spruce cutovers near the Town of Senneterre, Canada (48° N, 76° W). Soils of the region are classified mainly as Humo-Ferric Podzols (Soil Classification Working Group 1998) with a prominent mor humus layer. Three cutovers occurred on moist soils (spruce-moss ecotype) with a groundcover of feathermoss (*Pleurozium schreberi* (Brid.) Mitt); the other three sites occurred on well-drained soils (lichen-spruce ecotype) with a groundcover of fruticose lichens (*Cladonia* and *Cladina* spp.). Humus samples within each site were bulked and coarse-sieved (5 mm mesh). One subsample from each site was analysed for pH (1:10 = soil:water), for total P following sulphuric acid digestion (Kalra and Maynard 1991) and for total C, N and S by dry combustion (CHNS-O A1108 Fisons Instrument). Senescent black spruce needles and *Kalmia* leaves were collected on all sites, bulked according to species, and a litter subsample of each species was analyzed for total P, C, N and S as described above. Two more subsamples were extracted with acetone:water (70:30) and analyzed for total extractable phenolics by the Folin-Ciocalteu assay and for condensed tannins by the butanol-HCl assay (Waterman and Mole 1994; Preston et al. 1997). Total phenolics were standardized against tannic acid (Sigma Aldrich), whereas tannins were standardized against purified black spruce and *Kalmia* condensed tannins (Preston 1999).

Fresh humus subsamples from each site were weighed (15 g dry wt equiv.) into Mason jars and mixed with 3.0 g (dry wt) of chopped (3–5 mm) *Kalmia*:spruce litter mixtures (0:100, 25:75, 50:50, 75:25 and 100:0%). A non-amended (control) humus subsample was included for each site. Each litter mixture treatment from each site was replicated 5 times, giving a total of 180 jars (i.e., (5 Litter Mixtures + 1 Control) x 2 Ecotypes x 3 Sites/Ecotype x 5 Sampling Dates). Each jar was adjusted to 300% gravimetric moisture content and incubated in the dark at 20°C. Moisture content was re-adjusted with distilled water every week. After intervals of 2, 6, 13, 24 and 46 weeks, three jars of each treatment were destructively sampled. A 3 g (fresh wt) subsample was dried at 105 °C and weighed to determine moisture content, and the weight loss of humus in each jar was calculated. A second subsample (2 g) was analyzed for total phenolics and condensed tannins as described above, but standardized against purified balsam fir (*Abies balsamea* (L.) Mill.) tannin, whose response to the HCl-butanol reaction is intermediate between those of *Kalmia* and black spruce tannins. A third subsample (5 g) was extracted in 1.0 N KCl solution, filtered (0.5 μm) and analyzed for DIN ($NH_4^+ + NO_3^-$) by colorimetric procedures (Kalra and Maynard 1991). KCl extracts were also analyzed colorimetrically for DON following persulfate digestion (Cabrera and Beare 1993). A fourth subsample (12 g fresh wt) was used to derive indices of available C, which included basal respiration (BR) and microbial biomass (MB) (Bradley and Fyles 1995). BR was determined by measuring the amount of CO_2-C released following a 4 h incubation, using a model CP-2002 P Micro-GC (Chrompack, Middelburg) equipped with a TCD, with He as carrier gas. The same sample was amended with glucose (1000 μg C g^{-1}) and MB was then determined by substrate induced respiration (Anderson and Domsch 1978) using the same GC.

The effects of Ecotype, Sampling Date, Litter Mixture, and all interaction terms on the response variables were tested using three-way ANOVAs. When interactions terms were significant, the effects of one factor was tested in each level of the other factor using one-way ANOVAs. Means were separated using Ryan-Einot-Gabriel-Welsch *F*-tests. Orthogonal

polynomial contrasts (Gomez and Gomez 1984) were used to test for 1st and 2nd order trends in the relationship between % *Kalmia*:spruce litter and each response variable. Statistical analyses were performed using SPSS 11.01 (SPSS Inc., Chicago) software and $P< 0.05$ were considered significant.

Results

There were no significant differences in forest floor properties between ecotypes (Table 1). *Kalmia* litter had higher concentrations of N, tannins and phenolics, and a lower C:N ratio, than spruce litter (Table 1). Average mass loss of litter-amended forest floor material after 46 wks was 12%, but there were no significant differences between mixtures and ecotypes. Three-way ANOVAs showed a significant effect of Sampling Date on all variables, a significant effect of Ecotype on condensed tannins, total phenolics and MB, and a significant effect of Litter Mixtures on condensed tannins and total phenolics. There were, however, significant interactions between Ecotype and Sampling Date for DIN, between Ecotype and Litter Mixtures in controlling total phenolics, and between Sampling Date and Litter Mixtures in controlling condensed tannins, total phenolics, DON:DIN ratio, BR and MB. More specifically, condensed tannins increased with an increasing proportion of *Kalmia* litter (1st order trends; $P<0.001$), but decreased with sampling date (Fig. 1a,b). Similar trends ($P<0.04$) were observed for total phenolics (Fig. 1c,d) after 2, 6 and 13 weeks incubation. DIN and DON increased with incubation time (Fig. 2a,b) and DIN was higher in amended moss than amended lichen humus at week 46 (data not shown). The DON:DIN ratio was significantly higher in the spruce-moss ecotype and decreased with sampling date (Fig. 2c). At week 13, a positive linear trend ($P<0.001$) was found between the DON:DIN ratio and the proportion of *Kalmia* litter (Fig. 2c), mainly the result of higher DON with increasing *Kalmia* litter ($P=0.006$, Fig. 2b). A negative linear trend was found between BR and the proportion of

Kalmia litter at week 2 ($P<0.001$) and week 6 ($P=0.028$) (Fig. 2d), and between MB and the proportion of *Kalmia* litter at week 2 ($P<0.001$) (Fig. 2e).

Table 1. Chemical properties of forest floor and litter materials (forest floor: n=3 sites per Ecotype (1 SD); litter: pooled across sites before analysis).

Forest floor	**Lichen-spruce**	**Spruce-moss**
Total-N (mg g^{-1})	9.7 (1.1)	11.3 (1.3)
Total C (mg g^{-1})	450 (32)	432 (34)
C/N	46.5 (2.4)	38.9 (6.6)
Total P (mg g^{-1})	0.5 (0.1)	0.5 (0.1)
Total sulphur (mg g^{-1})	1.8 (0.2)	1.7 (0.1)
pH (1:10 = soil:water)	3.51 (0.09)	3.34 (0.12)
Leaf litter	**Black spruce**	***Kalmia***
Total C (mg g^{-1})	566	574
Total N (mg g^{-1})	3.9	8.4
C/N	144	68
Total sulphur (mg g^{-1})	0.87	0.85
Total P (mg g^{-1})	0.36	0.61
Condensed tannins (mg g^{-1})	44	235
Total phenolics (mg g^{-1})	93	237

Discussion

Although the N concentration of *Kalmia* leaf litter was more than twice that of spruce needles, there were no differences in mineral N released among treatments. It is unlikely that

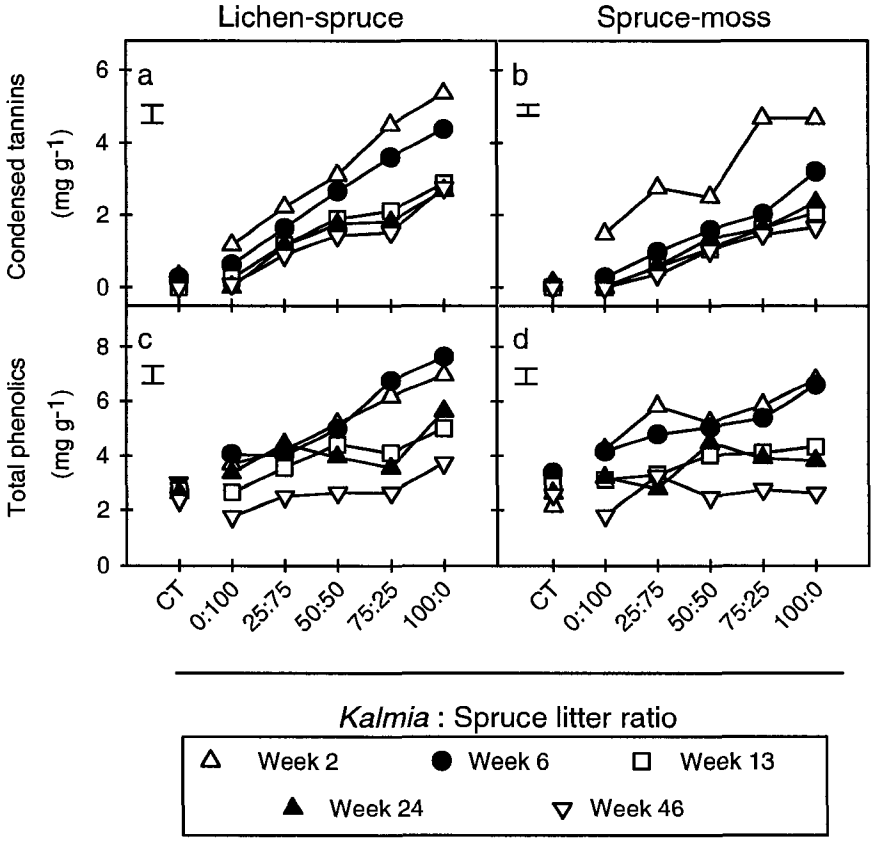

Figure 1. Extractable condensed tannins (a,b) and total phenolics (c,d) measured following the addition of *Kalmia*:spruce litter mixtures to forest floor material collected from three spruce cutover sites in both lichen-spruce and spruce-feathermoss ecotypes, and incubated 2, 6, 13, 24 and 46 weeks following the litter amendments. CT = non-amended control. Vertical line in top left corner of each frame designates the average standard deviation (n=3).

microbial immobilisation of mineral N was higher with increasing *Kalmia* litter, as trends in BR and MB would indicate the opposite. Therefore, that DON concentrations and the DON:DIN ratio both increased, at week 13, with an increasing proportion of *Kalmia* litter, corroborates the model proposed by Northup et al. (1995) whereby late-successional tannin-rich plant communities occurring on highly acidic soils manage to retain N in the ecosystem by precipitating soil proteins in recalcitrant organic forms. We observed this effect, however,

on the one sampling date, just as another study (Verkaik et al. 2006) had previously found higher DON:DIN ratios in tannin-amended soils to be ephemeral. The reason for this non-persistence may be the eventual precipitation of protein-tannin complexes in non-soluble

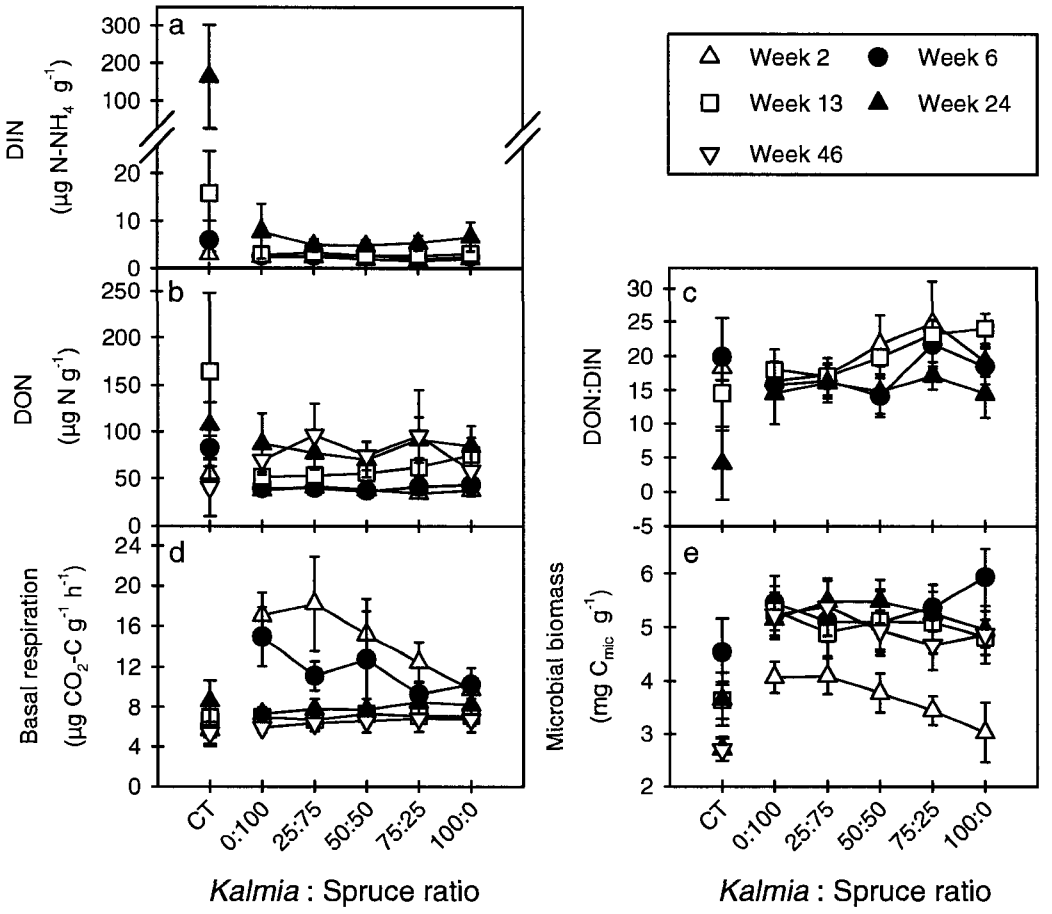

Figure 2. Pooled ecotype data for DIN (a), DON (b), DON/DIN ratios (c), basal respiration (d) and microbial biomass (e) following the addition of *Kalmia*:spruce litter mixtures to forest floor material collected from the six spruce cutover sites after 2,6,13, 24 and 46 weeks of incubation. Data of week 46 are omitted for DIN, and thus for DON:DIN, because significant differences between ecotypes were found at that week. CT = non-amended control. Bars represent standard deviations, which in some cases are smaller than the symbols.

forms, as these form resistant bonds with non-extractable humus particles (Nierop and Verstraten 2006). This is corroborated by the decreasing extractability of tannins and other phenolics as the incubation progressed. Although Northup et al.'s (1995) study revealed an important relationship that unified earlier work on leaf litter tannin production and plant nutrient uptake strategies, the transient nature of the DON:DIN ratio will likely preclude its usefulness as a robust indicator of ecological succession.

Acknowledgments

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CHAPITRE III

L'INHIBITION DES ENZYMES DU SOL PAR LES TANINS CONDENSÉS DE LA LITIÈRE PEUT DIRIGER LA STRUCTURE DE L'ÉCOSYSTÈME ET LES PROCESSUS : LE CAS DE *KALMIA ANGUSTIFOLIA*

Référence: Joanisse, G.D., Bradley, R.L., Preston, C.M. et Munson, A.D. (2007). Soil enzyme inhibition by condensed litter tannins may drive ecosystem structure and processes: the case of *Kalmia angustifolia*. New Phytol. *175*: 535-546.

Un point de vue majeur dans la littérature concernant l'effet des tanins sur le cycle des nutriments du sol est que les tanins rendent les nutriments non disponibles en les séquestrant dans des complexes récalcitrants à la décomposition (par exemple, des complexes proteines-tanins). Outre qu'on a démontré que l'azote des protéines complexées avec des tanins était très peu minéralisé dans les sols (Chapitre I), un autre mécanisme pas encore très bien exploré dans les sols (c'est-à-dire dans des conditions de sols) qui pourrait être important est que les tanins pourraient diminuer la disponibilité d'éléments simples (ammonium, phosphore et glucose) en inhibant les enzymes qui se retrouvent dans le sol responsables de leur relâchement de la matière organique. L'objectif principal était de vérifier l'hypothèse que les tanins relâchés par la litière de *Kalmia angustifolia*, suivant son envahissement sur les parterres de coupes, réduit l'activité des enzymes du sol, et sont alors important pour contrôler les processus de l'écosystème. De plus, compte tenu que l'épinette noire contient également des tanins condensés, il y a eu comparaison avec ceux du *Kalmia* pour leur capacité à inhiber certains enzymes du sol.

Plus précisément, le manuscrit présente des évidences que (1) les tanins purifiés inhibent les enzymes du sol, (2) qu'une augmentation de *Kalmia* dans des mélanges de litière de *Kalmia*:épinette en décomposition en microcosme engendre une diminution de l'activité enzymatique des sols provenant de deux types de sites (Lichen et Mousse), (3) et qu'une augmentation du couvert de *Kalmia* sur des sites était relié à une activité enzymatique du sol plus faible. Donc, cette étude est très intéressante, puisqu'elle permet d'établir des liens entre différents niveaux et est originale puisqu'elle explore un autre mécanisme, l'inhibition des enzymes par les tanins, comme en partie responsable de la faible disponibilité des nutriments dans les écosystèmes dominés par les éricacées.

Ma contribution à l'élaboration des expériences, la récolte des données, les analyses en laboratoire et la rédaction du manuscrit fut majeure. Comme pour les autres manuscrits présentés, la mise en place des différentes parcelles d'études CPRS a été réalisée par l'équipe du Dr. Alison Munson qui a également contribué à la révision et à la correction du manuscrit final. Pour les parcelles d'études de forêts non-coupées, elles ont été mise en place par Félix Boulanger (ancien étudiant à la maîtrise du Dr. Bill Shipley). La Dr. Caroline Preston a caractérisé les tanins condensés du *Kalmia* et d'épinette que j'ai purifiés et que j'ai utilisés dans la première expérience, en plus d'avoir participé à la correction et proposer de nombreuses suggestions lors de la rédaction de l'article. Pour certaines analyses de laboratoires, j'ai été aidé par les stagiaires Philippe Lebel et Jonathan Lafond. Le Dr. Bill Parsons a également révisé l'orthographe et la grammaire anglaise d'une première version du manuscrit. La rédaction du manuscrit a été réalisée avec la collaboration de mon directeur, Dr. Robert Bradley, qui m'a aidé à mettre de l'ordre dans mes idées. Les Dr. Preston et Munson ont révisé le manuscrit.

Summary

- *Kalmia angustifolia* is an ericaceous shrub that can rapidly spread on recently harvested boreal forest sites, causing a slow-down in soil nutrient cycling and reduced growth of spruce seedlings. We hypothesised that tannins released from *Kalmia* litter suppress soil enzyme activity, and are thus important in controlling ecosystem structure and processes.

- We tested the effects of different concentrations of tannins extracted from both *Kalmia* and black spruce foliage on enzyme activities of soil extracts. We then investigated the effects of various *Kalmia*:black spruce litter mixtures on soil enzyme activity. Lastly, we measured the correlation between *Kalmia* cover in the field and soil enzyme activity.

- Both tannin types suppressed β-glucosidase and acid phosphatase activities, and the magnitude of these effects was concentration-dependent. β-glucosidase and amidase activity decreased linearly with an increasing *Kalmia*:spruce litter ratio added to soil. A field survey of 24 sites revealed a negative relationship between % *Kalmia* cover and β-glucosidase activity.

- Collectively, results of the three experiments converge to support our claim that enzyme inhibition by litter tannins has evolved as an important mechanism controlling ecosystem processes and structure following *Kalmia* invasion on recently disturbed forest sites.

Introduction

The depolymerisation of complex soil organic matter by microbial extracellular enzymes, with the subsequent release of plant available nutrients, is a key factor driving biogeochemical cycles (Schimel & Bennett, 2004). Soil enzyme activity correlates with many soil properties that vary during ecological succession, such as moisture content (Criquet *et al.*, 2004), pH (Li *et al.*, 2006), nutrient availability (Sinsabaugh *et al.*, 1994; Decker *et al.*, 1999) and microbial community structure (Waldrop *et al.*, 2000; Marschner *et al.*, 2005), but the mechanisms underlying plant-induced changes in soil enzyme activity are largely unknown. Surprisingly, successional changes in leaf litter quality, which has been amply studied in the context of decomposition and nutrient cycling (Sinsabaugh *et al.*, 1994; 2002), have so far received little attention with regards to soil enzyme activity. Leaf litter could directly promote extracellular enzyme activity by induction (Martens *et al.*, 1992; Sinsabaugh *et al.*, 2002; Sall *et al.*, 2003), or reduce enzyme expression and activity through catabolite suppression (Kirk & Fenn, 1982; Dilly & Nannipieri, 2001). Fresh litter may indirectly affect soil enzyme activity by modifying soil pH, which can alter the conformation of soil enzymes. In the present study, we hypothesised that inter-specific differences in the quality and quantity of leaf litter tannins may also cause differential inhibition of some important soil enzymes.

Condensed tannins, also referred to as proanthocyanidins, are polymers of flavan-3-ols linked by C-C bonds. These secondary plant metabolites are believed to provide a chemical defence against herbivores, because they form stable complexes with proteins, thereby reducing the nutritional value of leaves (Appel, 1993). Many forest plants export large quantities of tannins in litterfall (Kraus *et al.*, 2003), where they may form stable cross-links with proteins and other organic compounds in the forest floor (Preston, 1999; Hernes *et al.*, 2001). Litter tannins are thus expected to reduce rates of litter decomposition (Driebe & Whitham, 2000)

and soil nutrient cycling (Bradley *et al.*, 2000b; Kraus *et al.*, 2004; Nierop *et al.*, 2006). Structural differences in chain length, hydroxylation pattern of the B-ring of the monomer units, and the stereochemistry of the links between monomers, could explain why tannins produced by different plants react differently in the environment (Kraus *et al.*, 2003).

Negative relationships between tannin concentrations and enzyme activities were demonstrated in several studies, but these studies either used generic (i.e., synthetic) tannins or were developed for non-soil systems (Goldstein & Swain, 1965; Sarkar & Burns, 1983). In natural systems, the interference of litter tannins with soil enzyme activity has been largely inferred through indirect evidence (e.g., Sivapalan & Fernando, 1983). The one study that did attempt to test this hypothesis (Fierer *et al.*, 2001) was unsuccessful in demonstrating a direct link between forest floor enzyme activity and litter tannin additions. The authors of that study suggested that the tannin concentrations they used were perhaps too low to produce substantial inhibitory effects. Given that they used a single incubation period (i.e., 20 d), it is also possible that they incorrectly timed their measurements. There is a need, therefore, to test the effects of litter tannins on soil enzyme activity over a range of concentrations and sampling periods, as well as to link results from controlled laboratory conditions to field observations.

In devising such an experiment, it is useful to work in a model system with accumulated literature in which the effects of litter tannin on soil nutrient cycling have regularly been evoked. For example, the role of leaf litter tannins and other metabolites on soil processes in conifer–ericad shrub communities has been amply studied and discussed (Mallik, 1995; Mallik, 2003). One such shrub, *Kalmia angustifolia* L. (hereafter referred to as *Kalmia*), is known to reduce the growth rate of commercially-valued black spruce (*Picea mariana* (Mill.) BSP) seedlings following stand disturbance (e.g., Peterson, 1965). *Kalmia* is an erect rhizomatous woody shrub with persistent leaves that can form dense thickets up to 1.5 m in

height. Black spruce regeneration on cutovers with high *Kalmia* cover (> 40%) normally requires major silvicultural interventions such as scarification and fertilisation to restore adequate growth rates (Thiffault *et al.*, 2004; Thiffault *et al.*, 2005). On sites with intermediate *Kalmia* cover (15–30%), forest succession may progress towards canopy closure, or may regress towards *Kalmia*-dominated heaths (Ruel *et al.*, 2004). The uncertain successional pathway on these intermediate sites presents a management challenge to foresters, and therefore, it is important that we understand the underlying mechanisms that determine *Kalmia* invasion. Bradley *et al.* (2000b) have shown that the addition of *Kalmia* tannins to forest floor humus reduces N mineralisation rates, and they proposed that this was perhaps due to the binding and sequestration of organic N sources that would otherwise be degraded. An extension of this argument is that tannins should also inhibit soil enzymes, in particular through formation of tannin-protein complexes.

We report on a study in which three experiments were performed to provide evidence that *Kalmia*-induced conifer reduced growth may result, to some extent, from reduced soil enzyme activities due to *Kalmia* litter tannins. The soil enzymes we tested are implicated in the carbon, nitrogen and phosphorus cycles (β-glucosidase, amidase and acid phosphatase respectively). In a first laboratory experiment, we tested the effects of different concentrations of tannins extracted from both *Kalmia* and black spruce foliage on the enzyme activities of soil extracts. In a second microcosm-based experiment, we looked at the effects of different ratios of *Kalmia* and black spruce litters on soil enzyme activity in order to simulate litter effects associated with different degrees of *Kalmia* cover in the field. In a third experiment, we measured the correlations between *Kalmia* cover in the field and soil enzyme activity.

Materials and Methods

Field sites

Our field sites were located in the boreal forest near the town of Senneterre, in the Abitibi region of Québec, Canada (ca. 48° N, 76° W). Soils of the region are classified mainly as Humo-Ferric Podzols (Blouin & Berger, 2001). Mean annual temperature is 0.5 °C and the average annual precipitation is 972 mm (Environment Canada, 2002). Plots (50 x 50 m) were established on 24 independent sites located within a 50 km radius. Sixteen sites had been harvested for black spruce 10 years previously; the other eight sites were non-harvested closed canopy spruce-feathermoss forest. Harvesting had been accomplished by "careful logging", which is referred to in Québec as CPRS (MRNFPQ, 2003). The practice of CPRS (Cut with Protection of Regeneration and Soils) requires that heavy machinery traverse no more than 25% of the harvested area, that crowns and branches remain on site, and that advanced regeneration of black spruce be maintained. Eight of the 16 cutover sites occurred on moist soils (mesic drainage class), where the groundcover consisted of a feathermoss mat (mainly *Pleurozium schreberi* (Brid.) Mitt); the other eight cutover sites occurred on dry soils (xeric drainage class), where the ground was carpeted with fruticose lichens (*Cladonia* and *Cladina* spp., mainly *Cladina rangifera*). The eight undisturbed forests were dominated by black spruce and the understory shrub community was comprised of various ericaceous shrub species including *Kalmia*, *Vaccinium* spp., *Rhododendron groenlandicum* (Oeder) Kron & Judd, and *Gaultheria procumbens* L. *Kalmia* dominated the shrub layer both in the undisturbed forest and in the cutovers.

Forest floor and leaf litter sampling

Cores (25 X 25 cm) of forest floor (F-H layer) material, 5-10 cm thick, were collected every 5 m along two 50 m transects established on each site during the first weeks of October 2002

and 2003. The cores within each site were pooled and sieved to pass a 5 mm mesh. The 24 bulked samples were transported on ice to the *Laboratoire d'écologie des sols – Université de Sherbrooke*, and stored at 4°C until analyses began two weeks later. On each CPRS site, plastic sheets were laid at the base of several *Kalmia* swards and black spruce trees in order to collect freshly senescent leaves and needles. The trees were shaken manually so as to increase needle drop. Leaves or needles were pooled across sites for use in Experiments 1 and 2 (described below).

Forest floor and leaf litter characteristics

Total N and P contents of the forest floor material from each site were determined colorimetrically using a Technicon Auto-analyser (Pulse Instrumentation, Saskatoon, SK), following the wet acid digestion of dried and finely ground subsamples. Forest floor pH was measured electrometrically from aqueous suspensions (soil:water = 1:10). Organic matter content was estimated by loss-on-ignition (Nelson & Sommers, 1996). Fresh subsamples (5 g dry mass equiv.) were extracted in aqueous 1.0 *N* KCl and analysed colorimetrically for NH_4^+ (salicylate–nitroprusside-hypochlorite) and NO_3^- (Cd reduction) concentrations using the Technicon Autoanalyser. Potential mineral N availability was assessed by incubating another set of fresh subsamples in the dark at 20 °C for 30 days, analysing mineral N and correcting for initial concentrations (i.e., net N mineralisation). In all cases, NO_3^- concentrations were below detection limits of our instrument (0.06 $\mu g\ ml^{-1}$) and therefore, are not reported in the paper. Forest floor samples collected in 2002 were also extracted in Bray-1 reagent (Kuo, 1996) and analysed colorimetrically for available-P (vanado-molybdophosphoric acid assay) using the Technicon Autoanalyser.

Senescent *Kalmia* leaves and spruce needles were analysed for total N and total P as described above. Total C content was determined by high-temperature dry combustion,

followed by infrared detection using a Model CR12 Carbon Determinator (Leco Instruments, Mississauga, ON). Condensed tannins were analysed colorimetrically after hydrolysis with butanol/HCl using the proanthocyanidin assay (Preston *et al.*, 1997) and standardised against condensed black spruce and *Kalmia* tannins that had been purified according to Preston (1999). Total phenolics were determined by rehydrating 0.4 mL aliquots of dry acetone-water extracts with 1.0 mL distilled water, 0.5 mL Folin-Ciocalteu reagent (Sigma), 2.5 mL of aqueous Na_2CO_3 (20% w/v) and reading solution absorbance (750 nm) on a spectrophotometer standardised against tannic acid (Sigma) (Waterman & Mole, 1994).

Measuring enzyme activity

The activities of β-glucosidase and acid phosphatase were measured using microplate-based fluorimetric assays. β-glucosidase is an important glycosidase in soils, which catalyses the hydrolysis of β-d-glucoside-bonds found in cellobiose and similar carbohydrates. This enzyme is involved in the final step of cellulose degradation that provides simple sugars for soil microorganisms. Acid phosphatase is a phosphomonoesterase that catalyses the hydrolysis of both esters and anhydrides of phosphoric acid (Schmidt & Laskowski, 1961). The respective substrates for the two assays were 4-MUB-β-d-glucoside and 4-MUB-phosphate. MUB is 4-methylumbelliferone, which fluoresces once the enzyme has cleaved the attached substrate (Marx *et al.*, 2001). Briefly, 1 g (dry mass equiv.) subsamples of forest floor material were mixed with 50 mL of sterile distilled water, 1.5 mL of toluene to inhibit microbial activity (Tabatabai, 1994) and sterile glass beads (3-mm dia.). The mixtures were shaken on an orbital shaker (350 rpm for 5 min). For each enzyme, triplicate 20 μL aliquots of soil suspension were pipetted into three microplate wells to which were added 20 μL of MES (2-[N-Morpholino]ethanesulfonic acid) solution and 160 μL of 100 μM substrate/MES solution, thus yielding a final substrate concentration of 80 μM per microplate well. Plates were incubated at 30°C for 10 min, shaken to homogenise the reaction medium, and

fluorescence measurements were taken immediately (t = 0) and at 10 min intervals for 60 min (t = 10, 20, 30, 40, 50 and 60), using a microplate fluorimeter (MODEL FL600, Bio-Tek Instruments, Winnoski, VT). These repeated readings were performed to ensure that the increase in fluorescence was linear across the 60 min measurement period. Enzyme activity for each assay was calculated as the final (t = 60) reading minus the initial (t = 0) reading. The resulting data (fluorescence units/min) were converted into nmol of MUB min^{-1} g^{-1} soil with the use of a standard calibration curve. A standard curve was generated for each forest floor sample by transferring eight 20 μL aliquots of soil suspension into eight microplate wells, adding eight concentrations of MUB in the 0–1000 pM range, and measuring fluorescence. We included a control well to correct for substrate auto-hydrolysis (background fluorescence) using 20 μL of distilled water with 3% toluene and no soil suspension. MES solution was added to bring the volume of standards and blank wells to 200 μL.

Amidase is an enzyme that catalyses the deamination of aliphatic amides to monocarboxylic acids and ammonia. Amidase activity was determined by measuring the production of NH_4^+ following incubation with formamide (Tabatabai, 1994). Fresh forest floor material (2 g dry mass equiv.) was amended with 9 mL of 0.1 M THAM (tris(hydroxymethyl)amino-methane) solution, 0.2 mL of toluene and 1 mL of 0.1 M formamide, and incubated for 2 h at 37°C. A second forest floor subsample was incubated in the same way with distilled water instead of formamide. We also prepared formamide blanks, which contained no forest floor material, to control for formamide degradation in the absence of soil. Following incubation, 39.8 mL of 2*N* KCl was added to each suspension, which was then filtered through 0.5-mm screen to remove debris. Ammonia-N was immediately determined by titration following steam distillation (Tecator Kjeltec System 1002, Högonäs, Sweden) of the KCl extracts. The value of each sample was corrected for its corresponding blank (distilled water) and formamide blank (no soil).

Experiment 1: Effects of Kalmia and spruce tannins on soil enzyme activities

Purified condensed tannins were prepared from fresh *Kalmia* and black spruce litters, as outlined in Preston (1999). General structural information of the tannins was obtained from solution ^{13}C NMR spectroscopy (Czochanska *et al.*, 1980; Ayres *et al.*, 1997). Briefly, *Kalmia* tannins consisted of 80% procyanidin (PC) units with 82% *cis* stereochemistry, an average chain length of 2.3 units, and 73% of terminal units were *trans*. Black spruce tannins consisted of 90% PC units with 82 % cis stereochemistry, an average chain length of 6.0 units, and 69% of terminal units were *trans*. We tested the effects of incremental additions of these two tannins on β-glucosidase and acid phosphatase activities (as described above) in soil solutions from the 24 sites. Following the addition of 20 μL of soil extracts to each well, we added 20 μL of MES solution carrying *Kalmia* or black spruce tannins so as to yield final tannin concentrations of 0, 0.025, 0.05, 0.10 and 0.20 mg mL^{-1}. Based on our estimate of *Kalmia* litter tannin production, the highest of these concentrations is thought to be less than episodic litter tannin additions in the field. In producing the MUB calibration curves, soil extracts plus tannins were used to correct for the quenching effects of tannins. In order to adequately compare results from the 24 field sites, data were transformed to a relative "percent enzyme inhibition" (PEI) value, calculated as follows:

$$PEI = \frac{\text{(control enzyme activity – treatment enzyme activity)}}{\text{(control enzyme activity)}} * 100\% \qquad [1]$$

Experiment 2: Effects of Kalmia:spruce litter mixtures on soil enzyme activities

In a microcosm study, we simulated the litter effects associated with *Kalmia* invasion on black spruce cutovers by amending forest floor humus with *Kalmia*:spruce litter mixtures of

varying proportions (0:100, 25:75, 50:50, 75:25 and 100:0 %). The litters were chopped (3-5 mm) so as to restore comminution effects imparted by the soil fauna that could have been lost during the collection, sieving and transport of the forest floor material. In order to verify that these ratios were realistic, a crude estimate of the relative contributions of *Kalmia* and black spruce to total litterfall on regenerating cutovers was calculated from the mass of senescent *Kalmia* leaves and spruce needles collected, May to October 2003, from 15 randomly positioned litter traps (0.25 m^2) on 11 CPRS sites. We used humus collected from three CPRS sites of each ecotype (i.e., spruce-feathermoss and lichen-spruce) that displayed similar (i.e., intermediate) *Kalmia* cover (20-25%). The humus samples were weighed (15 g dry mass equiv.) into 500 mL Mason jars and amended with 3.0 g of dried *Kalmia*:spruce litter mixtures. A non-amended (i.e., control) humus sample was included for each of the six sites. Each of the 12 treatments [2 ecotypes x (5 litter mixtures + control]) was replicated 12 times. Gravimetric moisture content in each microcosm was adjusted to 300%, the jars were sealed with a polyethylene film to minimise water loss and maintain gas exchange, and incubated in the dark at 20°C. Moisture content was re-adjusted to 300% every 2 weeks. After 2, 6, 13, and 46 weeks, three jars from each treatment were destructively sampled in order to measure β-glucosidase and acid phosphatase activities, as described above. Amidase activity was measured after 6, 13 and 46 weeks. Before each sampling, about 3 g (fresh mass) of incubation mixture was oven-dried at 105°C to determine its moisture content.

Experiment 3: Relationship between Kalmia cover and enzyme activities

In July 2003, *Kalmia* cover on the 24 field sites was estimated from 20 quadrats (0.5 x 0.5 m) spaced 5 m apart along two transects (Kent & Coker, 1992). The activities of β-glucosidase and acid phosphatase in forest floor samples from each quadrat were measured according to the protocols described above. Values were regressed against percent *Kalmia* cover and other soil properties previously described.

Statistical analyses

In Experiment 1, we tested the effects of site type (harvested spruce-feathermoss *vs.* harvested lichen-spruce *vs.* non-harvested spruce moss), tannin type (spruce *vs. Kalmia*), tannin concentration, and all interaction terms, on the activity of forest floor enzymes using three-way ANOVA. When significant interaction terms were found, we subsequently sorted the data by each experimental factor and re-analysed using one-way ANOVA. When significant effects were found, means were separated using post hoc pairwise comparisons (Ryan-Einot-Gabriel-Welsch *F*-tests). We compared each tannin concentration to the control soil (i.e., PEI = 0) using Student's *t*-tests.

In Experiment 2, we compared initial forest floor characteristics of the two harvested ecotypes using Student's *t*-test. As in Experiment 1, we tested the effects of ecotype, sampling date, litter mixtures, and all interaction terms, using three-way and one-way ANOVAs followed by post hoc pairwise comparisons when significant effects were found. Single degree-of-freedom (*df*) orthogonal contrasts were used to compare the effect of each litter mixture within each site type pooled across all dates, to the enzyme activities in the corresponding control. We used orthogonal polynomial contrasts (Gomez & Gomez, 1984) to test for 1^{st} and 2^{nd} order trends in the relationship between % *Kalmia*:spruce litter and enzyme activity; a significant 1^{st} order (linear) trend indicates an effect of litter mixtures whereas a significant 2^{nd} order (quadratic) trend indicates an interaction between the two litter types during the incubation (Fyles & Fyles, 1993). When 1^{st} order trends were found, analysis of covariance (ANCOVA) was used to test for significant differences in slopes and intercepts between sampling dates.

In Experiment 3, we tested the effect of site type on forest floor properties using one-way ANOVA. Pearson correlation coefficients were calculated to relate enzyme activities to % *Kalmia* cover and forest floor properties. Stepwise multiple linear regressions were performed using enzyme activity as the dependent variable, and % *Kalmia* cover plus one forest floor property as independent variables. The selection of independent variables was performed by introducing a threshold F value of 2.5, and used only variables that were not significantly correlated with each other.

Statistical analyses were performed using SPSS 11.01 (SPSS Inc., Chicago, IL.) software. Prior to analyses, we verified that the data conformed to assumptions of normality and homogeneity of variance; data were Ln-transformed when necessary to meet these assumptions. The level of significance for all tests was set to $P \leq 0.05$.

Results

Experiment 1: Enzyme inhibition by pure tannins

Three-way ANOVA showed a significant effect of tannin type ($F_{1,167} = 8.15$, $P = 0.005$), tannin concentration ($F_{3,\ 167} = 35.8$, $P < 0.001$), and of the interaction between these two factors ($F_{3,167} = 5.48$, $P = 0.001$), on β-glucosidase activity. Subsequent one-way ANOVA tests revealed significant effects of tannin concentration on PEI of β-glucosidase for both spruce ($F_{3,91} = 8.40$ $P < 0.001$) and *Kalmia* ($F_{3,92} = 33.71$, $P< 0.001$) tannins. Post hoc comparisons revealed significant PEI at all four spruce tannin concentrations, but only at the three highest *Kalmia* tannin concentrations (Fig. 1a). At the lowest concentration, PEI was significantly ($t_{48}=3.818$, $P<0.001$) higher with spruce tannins than with *Kalmia* tannins. At

the highest concentration, β-glucosidase activity was about 60% lower than the control, for both tannin types (Fig. 1a).

Acid phosphatase activity was significantly affected by site type ($F_{2, 167} = 4.98$, P = 0.008), tannin type ($F_{1, 167} = 105.8$, P < 0.001), tannin concentration ($F_{3, 167} = 83.4$, P < 0.001), and by a (tannin type x tannin concentration) interaction ($F_{3, 167} = 15.6$, P < 0.001). More specifically, average PEI was significantly higher on lichen-spruce cutovers (25%) than on harvested (18%) and non-harvested (17%) spruce-feathermoss sites (data not shown). One-way ANOVA revealed significant effects of tannin concentration on PEI of acid phosphatase for both spruce ($F_{3, 91} = 15.28$, P <0 .001) and *Kalmia* ($F_{3, 92} = 74.76$, P < 0.001) tannins. Post hoc comparisons revealed significant PEI at all four spruce tannin concentrations, but only at the two highest *Kalmia* tannin concentrations (Fig. 1b). For *Kalmia* tannins, PEI of acid phosphatase at the lowest concentration was significantly lower than the control, indicating a stimulatory effect. At the three lowest concentrations, PEI was significantly higher with spruce than with *Kalmia* tannins ($t_{46} > 4.9$, P < 0.001). At the highest concentration, acid phosphatase activity was about 40% lower than the control, for both tannin types (Fig. 1b).

Experiment 2: Enzyme inhibition with an increasing proportion of Kalmia litter

Chemical properties of the humus and litter material used in this experiment are shown in Table 1. There were no significant differences in forest floor properties between the two ecotypes. Compared to spruce needle litter, *Kalmia* leaf litter had higher concentrations of N (+214%), P (+169%), total phenolics (+254%) and condensed tannins (+537%).

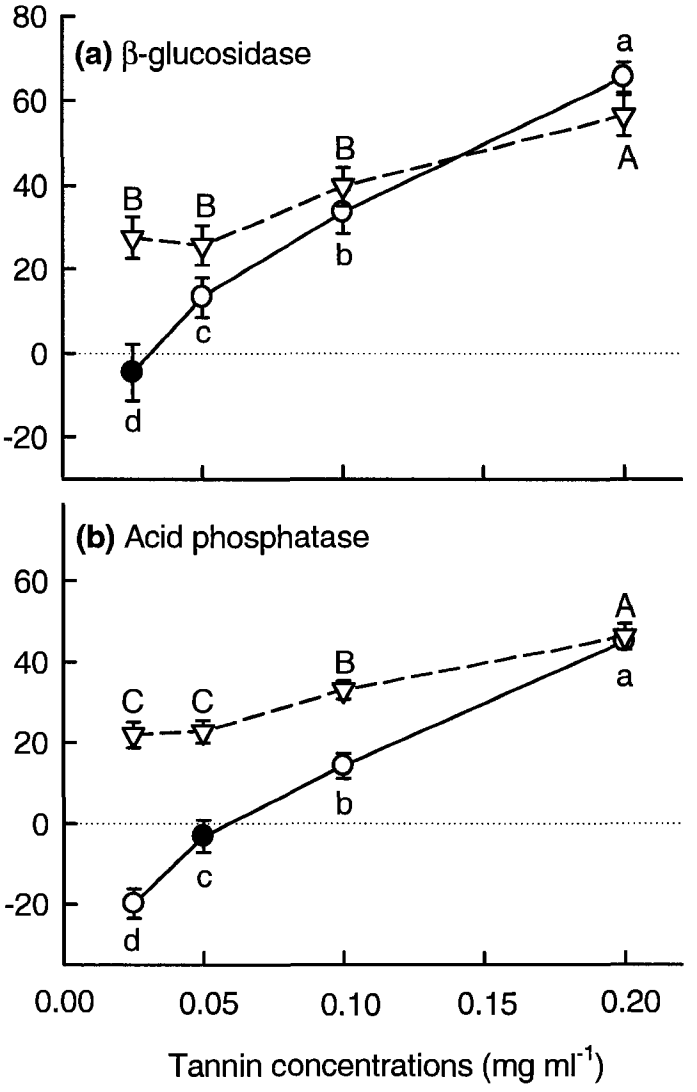

Figure 1. Percent inhibition of (a) β-glucosidase and (b) acid phosphatase in forest floor water extracts following the addition of *Kalmia* (circles) and black spruce (triangles) tannins. Each point represents the mean (± 1 SE) of 24 plots. Circles with different lower-case letters, and triangles with different capital letters, represent significantly different ($P < 0.05$) PEI values according to Ryan-Einot-Gabriel-Welsch *F*-tests. Filled symbols designate PEI values that do not differ significantly ($P > 0.05$) from the control treatment according to Student's t-tests.

From the litter collected on 11 CPRS sites (i.e., 165 litter traps) from May to October, we estimated a range of *Kalmia* leaf litterfall of 6–43 kg ha^{-1}, spruce litterfall of 1–32 kg ha^{-1}, and a *Kalmia*:spruce litter ratio of 0.20–0.97. We found a significant ($r^2 = 0.86$, $F_{1,9} = 54.0$, $P < 0.001$) relationship between *Kalmia* cover and *Kalmia* leaf litterfall, but not between *Kalmia* cover and spruce litterfall (Fig. 2a). *Kalmia* cover was significantly ($r^2 = 0.37$, $F_{1,9} = 5.25$, $P = 0.047$) related, however, to the *Kalmia*:(*Kalmia*+spruce) litter ratio (Fig. 2b).

Three-way ANOVA revealed a significant effect of sampling date ($F_{3,80} = 15.2$, $P < 0.001$) and litter mixture ($F_{4,80} = 12.8$, $P < 0.001$) on β-glucosidase activity. More specifically, β-glucosidase activity at weeks 2 and 6 was significantly lower than at weeks 13 and 46 (Fig. 3a,b). Polynomial contrast analysis revealed a significant linear trend (1^{st} order, $F_{1,115} = 38.828$, $P < 0.001$) for all sampling dates, which indicated that β-glucosidase activity decreased linearly with an increasing proportion of *Kalmia* litter. ANCOVA revealed that slopes did not differ significantly across sampling dates ($F_{3,112} = 0.789$, $P = 0.50$), but the intercepts did ($F_{3,115} = 17.03$, $P < 0.0001$). After pooling the data across all sampling dates and ecotypes, single *df* orthogonal contrasts revealed that β-glucosidase activity in the non-amended control was significantly lower than in the 75% and 100% black spruce mixtures ($P = 0.014$ and $P = 0.050$, respectively), but significantly higher than in the 0% black spruce (i.e., 100% *Kalmia*) litter treatment ($P = 0.037$).

Three-way ANOVA revealed a significant effect of sampling date ($F_{3,80} = 13.79$, $P < 0.001$) and site type ($F_{1,80} = 37.2$, $P < 0.001$) on acid phosphatase activity, but no effect of litter mixture. More specifically, phosphatase activity was significantly higher in weeks 13 and 46 than in weeks 2 and 6, and significantly higher in spruce-feathermoss than in lichen-spruce forest floor material (Fig. 3c,d). Orthogonal contrasts revealed significantly ($P < 0.02$) lower acid phosphatase activity in litter-amended lichen-spruce humus than in the non-amended control for weeks 2 and 6.

Table 1. Chemical characteristics of forest floor and litter material used in Experiment 2; (forest floor, n=3/site-type (1 SD); litter pooled over 16 sites).

Forest floor	Lichen-spruce (n=3)	Spruce feathermoss (n=3)
Total-N (mg N g^{-1})	9.7 (1.1)	11.3 (1.3)
Total-C (mg C g^{-1})	450 (32)	432 (34)
C/N ratio	46.5 (2.4)	38.9 (6.6)
Total-P (mg P g^{-1})	0.5 (0.1)	0.5 (0.1)
Extractible-N (μg NH_4^+-N g^{-1})	2.1 (0.7)	1.6 (0.4)
Bray 1 extractible-P (μg PO_4^{2-}-P g^{-1})	4.9 (2.2)	3.5 (1.5)
pH (1:10 = soil:H_2O)	3.5 (0.1)	3.3 (0.1)
Needle/leaf litter	**Black spruce**	***Kalmia***
Total-N (mg N g^{-1})	3.92	8.41
Total-C (mg C g^{-1})	566	574
C/N	144.4	68.2
Total-P (mg P g^{-1})	0.36	0.61
Total phenolics (mg tannic acid g^{-1})	93.5	237.3
Condensed tannins (mg g^{-1})	43.8	235.4

Three-way ANOVA revealed a significant effect of sampling date ($F_{2,60} = 58.3$, $P < 0.001$), litter mixture ($F_{4,60} = 2.72$, $P = 0.038$), and (ecotype x sampling date) interaction ($F_{2,60} = 7.60$, $P = 0.001$) on amidase activity. More specifically, one-way ANOVAs and post hoc comparisons revealed similar effects of sampling date on amidase activity within each site

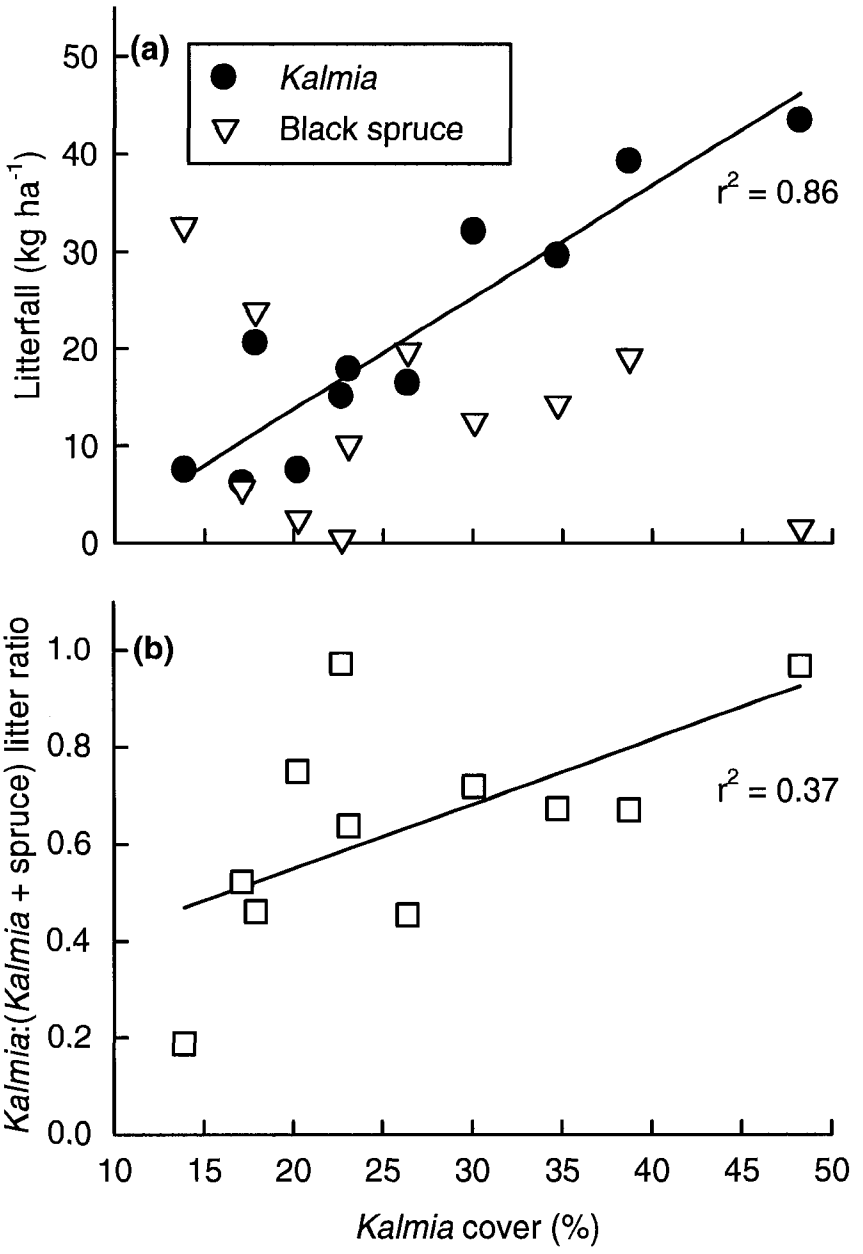

Figure 2. Relationship between % *Kalmia* cover and (a) the amount of *Kalmia* and spruce leaf litter collected from May to October 2003 on 11 CPRS cutover sites, and (b) the *Kalmia*:(*Kalmia* + spruce) leaf litter ratio. Each point represents the average of 15 litter traps. Regression line in top panel is for *Kalmia* leaf litter only.

type (lichen-spruce: 46 wk < 6 wk < 13 wk; spruce-feathermoss: 46 wk < 6 wk = 13wk). Orthogonal polynomial contrast revealed a significant linear trend (1st order, $F_{1,85} = 4.43$, P = 0.038) in the pooled data set, indicating a general decrease in amidase activity with an increasing proportion of *Kalmia* litter (Fig. 3e,f). This trend was consistent, however, for only three of the six (ecotype x sampling date) combinations (i.e., lichen-spruce at 6 wk; spruce-feathermoss at 13 and 46 wk). ANCOVA revealed that slopes did not differ significantly across sampling dates ($F_{2,84} = 0.205$, P = 0.82), but the intercepts did ($F_{2,86} = 59.49$, P < 0.0001). Single *df* orthogonal contrasts revealed that amidase activity was significantly lower (P < 0.05) in the non-amended control than in the amended soil for each (ecotype x sampling date) combination. Student's t-test indicated significantly higher amidase activity in lichen-spruce than in spruce-feathermoss humus on week 13.

Experiment 3: Enzyme activities and % Kalmia cover in the field

Average forest floor properties of the three site types (n = 8 per site type) used in the field study are compared in Table 2. Briefly, organic matter, soil moisture, NH_4^+-N and net N mineralisation were significantly higher in non-harvested spruce-feathermoss than in harvested lichen-spruce and/or spruce-feathermoss sites. Forest floor pH was significantly higher in harvested lichen-spruce than in the other two site types.

Correlation coefficients between forest floor properties and enzyme activities are given in Table 3. Percent *Kalmia* cover was negatively correlated with β-glucosidase activity, but not with phosphatase activity. Organic matter and moisture were positively correlated with β-glucosidase activity. Organic matter, moisture and NH_4^+-N were positively correlated, and pH negatively correlated, with acid phosphatase activity (Table 3).

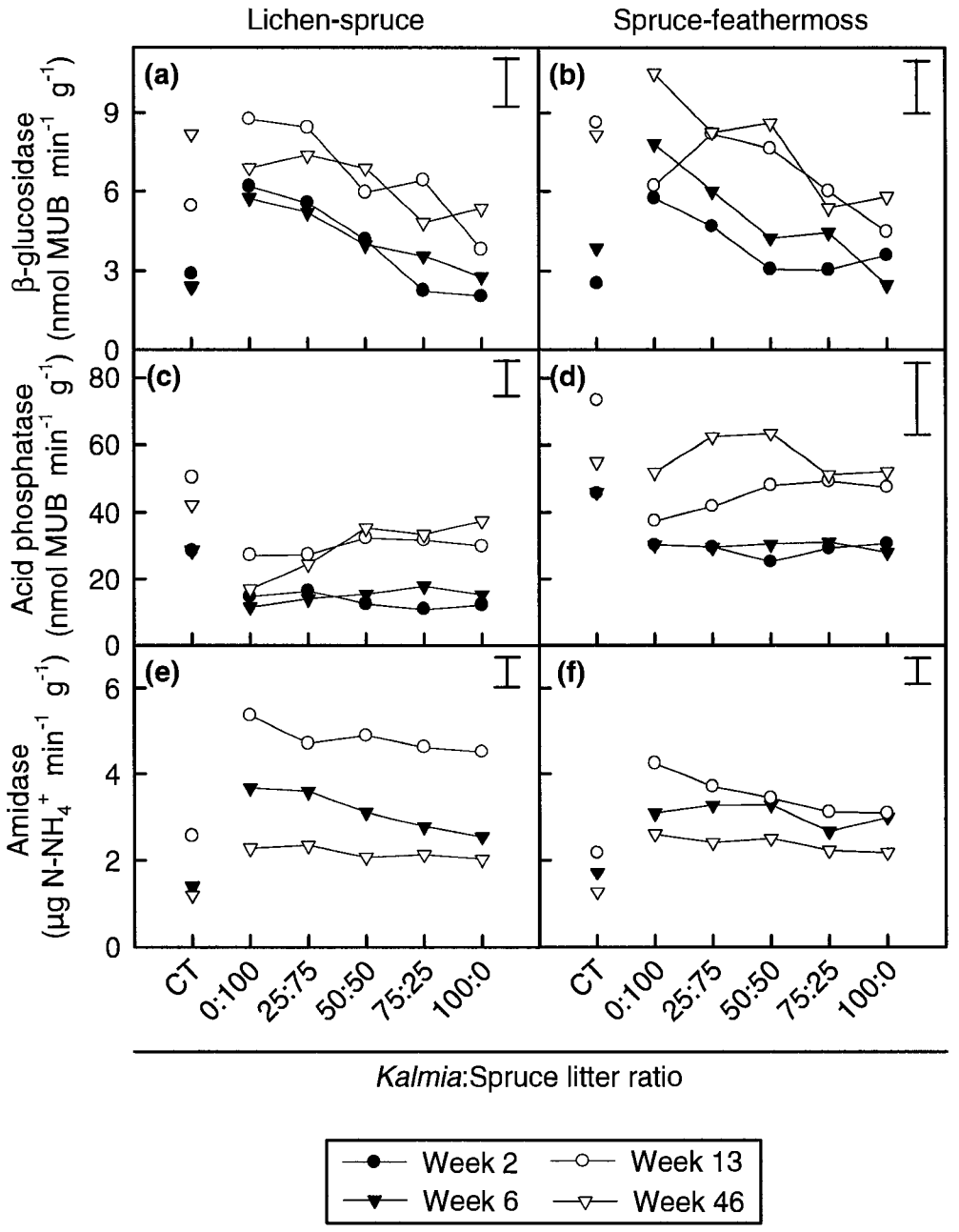

Figure 3. Enzyme activities in forest floor water extracts following the addition of *Kalmia*:spruce litter mixtures. Forest floor material was collected from three CPRS cutover sites in both lichen-spruce and spruce-feathermoss ecotypes, and incubated 2, 6, 13 and 46 weeks following the litter amendments. CT = unamended control. Vertical bars in top right corner of each panel = average SE.

Table 2. Percent *Kalmia* cover and chemical/biochemical properties of forest floors sampled in Experiment 3. Values represent the average of eight sites per site-type (parentheses = 1 SD). Values on the same line followed by a different lower-case letter are significantly different ($P < 0.05$) based on Ryan-Einot-Gabriel-Welsch post hoc *F*-tests.

Properties	Harvested lichen-spruce	Harvested spruce-feathermoss	Non-harvested spruce-feathermoss
Kalmia cover (%)	19.6 (8.1) a	28.2 (11.9) a	20.5 (9.8) a
Total-N (mg N g^{-1})	12.2 (5.5) a	12.3 (3.4) a	12.2 (2.2) a
Total-C (mg C g^{-1})	430 (33) b	465 (69) ab	514 (29) a
C/N ratio	39.8 (12.3) a	39.7 (8.1) a	42.9 (6.6) a
Total-P (mg P g^{-1})	0.5 (0.1) a	0.5 (0.1) a	0.5 (0.1) a
Organic matter (%)	71.9 (9.2) b	75.6 (4.7) b	83.7 (5.1) a
Extractible-N (μg NH_4^+-N g^{-1})	2.5 (0.2) ab	2.2 (0.5) b	4.1 (2.9) a
Net N mineralisation (μg NH_4^+-N g^{-1} $30d^{-1}$)	2.7 (1.8) ab	1.6 (0.9) b	22.7 (36.9) a
pH (1:10 = soil:water)	3.5 (0.2) a	3.3 (0.1) b	3.3 (0.1) b
Moisture (gravimetric %)	273 (44) b	256 (51) b	345 (39) a
Acid phosphatase (nmol MUB min^{-1} g^{-1})	18.8 (7.6) b	24.8 (15.9) b	51.7 (22.9) a
β-glucosidase (nmol MUB min^{-1} g^{-1})	2.6 (1.3) a	1.7 (1.0) a	2.4 (0.7) a

Table 3. Pearson's correlation coefficients linking soil enzyme activities to % *Kalmia* cover and selected soil variables. Values shown in bold are statistically significant ($P < 0.05$).

	β-glucosidase	Acid phosphatase
Kalmia cover (%)	**-0.35**	0.11
Total-N (mg N g^{-1})	0.15	0.19
C/N ratio	-0.20	0.04
Total-P (mg P g^{-1})	0.32	0.18
Organic matter (%)	**0.47**	**0.60**
Extractible NH_4^+-N (μg N g^{-1})	0.29	**0.42**
Net N mineralisation (μg NH_4^+-N g^{-1} $30d^{-1}$)	0.16	0.26
Moisture (gravimetric %)	**0.41**	**0.77**
pH (1:10 = soil:H_2O)	-0.15	**- 0.61**

The best-fitting bivariate linear regression model for β-glucosidase activity included percent *Kalmia* cover and organic matter, with standardised partial regression coefficients of -0.313 and 0.436, respectively ($r^2 = 0.312$, $F_{2,21} = 4.763$, $P = 0.020$). A 3-D mesh plot representing this regression is shown in Fig. 4. Since we were mainly interested in the effect of *Kalmia* cover on enzyme activity, no regression model is presented for acid phosphatase activity.

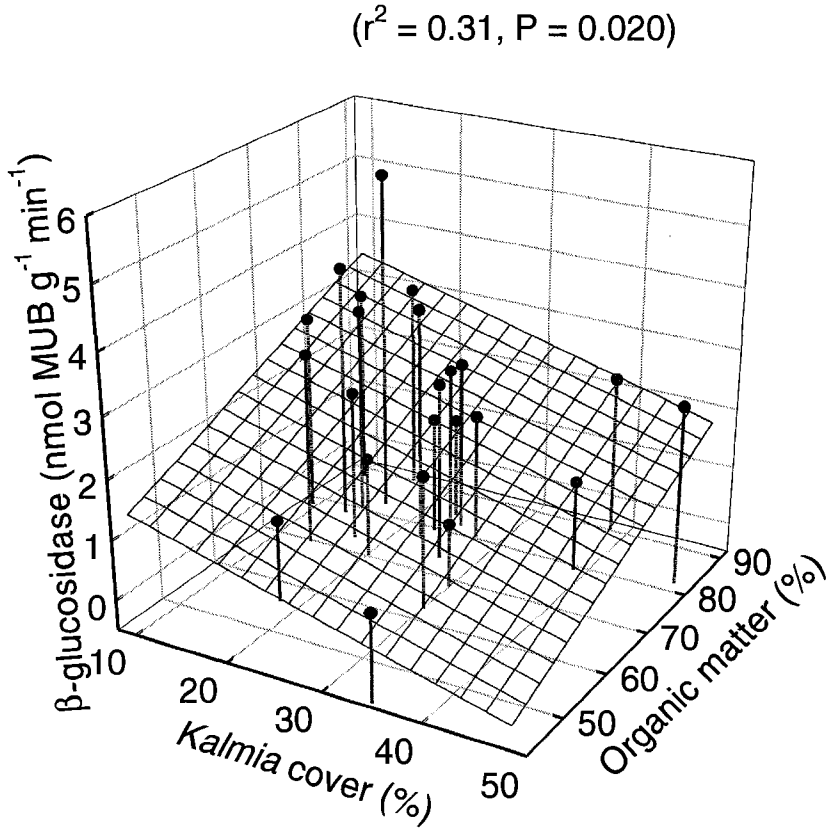

Figure 4. Relationship between β-glucosidase activity and the combined effects of *% Kalmia* cover and soil organic matter content. The grid-plane represents values predicted by the best-fitting bivariate regression equation.

Discussion

Enzyme inhibition is controlled by both the quality and quantity of litter tannins

In contrast to previous studies that have mostly used commercially-produced tannins (e.g., Yu *et al.*, 2000), enzymes (e.g., Benoit & Starkey, 1968; Vuorinen & Saharinen, 1996) or

both (e.g., Goldstein & Swain, 1965; Rao & Gianfreda, 2000), *in vitro* assays used in the present study measured the potential of condensed tannins extracted from fresh litters to reduce the activity of enzymes released naturally into soil solution. Results can be used, therefore, to directly infer the ecological importance of tannins on soil enzyme activity in *Kalmia*–black spruce ecosystems. Given that these forest floors are likely to be dominated by fungal biomass due to their low pH and high lignin (i.e., the operationally-defined acid unhydrolyzable residue fraction from proximate analysis) content (Preston *et al.*, 1997; Blagodatskaya & Anderson, 1998; Pennanen *et al.*, 1999), and because fungi mainly produce extracellular enzymes (Kirk & Farrell, 1987; Romani *et al.*, 2006), we are confident that our results were not biased by artefacts arising from experimental conditions that enabled the measurement of extracellular enzymes and can, therefore, be extrapolated to field situations.

In Experiment 1, the fact that enzyme inhibition at the lower tannin concentrations was higher for spruce than for *Kalmia* seems contrary to expectation. Estimates based on foliar tannin concentrations, *Kalmia* leaf litterfall and forest floor mass suggest, however, that even the highest *Kalmia* tannin addition rate used in this experiment was lower than the annual leaf litter tannin input in the field under moderate *Kalmia* cover. Given that the inhibitory effect of both tannin types remained concentration-dependent over the entire range of concentrations tested in Experiment 1, and given the higher concentration (> 5x) of condensed tannins in *Kalmia* than in spruce litter, we expect that even a moderate cover of *Kalmia* in the field will significantly reduce soil enzyme activities and ultimately alter ecosystem structure and processes.

It is curious that *Kalmia* tannins actually stimulated acid phosphatase activity at the 25 $\mu g\ ml^{-1}$ concentration, and we can only speculate on the causes. For example, Al^{3+} ions may also bind to, and inhibit, soil enzymes (Domenech *et al.*, 1992), but the hydrated forms of this ion may, in turn, be sequestered by some tannins (Appel, 1993; Kaal *et al.*, 2005). Thus, at low

concentrations, *Kalmia* tannins could increase acid phosphatase activity by preferentially binding to other enzyme inhibitors, whereas above some threshold value these tannins are directly inhibiting the enzymes. Fierer *et al.* (2001) found increased β-glucosidase activity in a soil developed under alder (*Alnus tenuifolia*), following the addition of low molecular weight tannins such as those produced by *Kalmia*, and an opposite effect with the addition of high molecular weight tannins such as those produced by black spruce. They ascribed these differences to low molecular weight tannins acting as C sources and thereby stimulating microbial activity. However, this reasoning cannot apply to our study, as the addition of toluene effectively inhibits microbial activity during the assay period (Frankenberger & Johanson, 1986). Given the high concentrations of tannins that naturally occur in *Kalmia* litter, we believe that the increase of acid phosphatase activity observed at the 25 $\mu g\ ml^{-1}$ tannin concentration may be of modest importance.

An increase in Kalmia cover and litterfall results in lower soil enzyme activity

Our microcosm study used forest floor material that had been collected from sites with intermediate *Kalmia* cover (20-25%). These sites are the least predictable in terms of forest succession, and amending the forest floor material with *Kalmia*:spruce litter mixtures of varying proportions was intended to simulate the litter effect associated with the progressive invasion of *Kalmia* on these sites. The litter-to-humus ratio of 0.2 that was used represents realistic field values (e.g., Titus et al., 1995; Inderjitt and Mallik, 1996). As experimental conditions were controlled, we effectively isolated the effect of litter mixtures on soil enzyme activity.

The addition of fresh organic material can either increase (Martens *et al.*, 1992; Acosta-Martinez *et al.*, 1999) or decrease (e.g., Kirk & Fenn, 1982) soil enzyme activities.

Accordingly, the addition of fresh litter in our study had both stimulatory and inhibitory effects on soil enzymes. Irrespective of this, and more importantly, our results confirmed that increasing the relative amount of *Kalmia* leaf litter reduced the activity of two enzymes, and had no effect on the third. Lower soil enzyme activity should favour the competitive ability of *Kalmia* owing to ericoid mycorrhizal root symbionts (Massicotte *et al.*, 2005) that are ostensibly capable of metabolising and absorbing nutrients from complex organic matter (Bending & Read, 1996; Bradley *et al.*, 1997; Read *et al.*, 2004). Thus, our results suggest that the establishment of *Kalmia* on forest cutovers could trigger a positive feedback loop involving litterfall tannin production, soil enzyme inhibition, a decrease in forest floor nutrient mineralisation rates, and an increase in the competitive ability of *Kalmia*. Bradley *et al.* (1997, 2000b) provided evidence that sites with inherently low nutritional quality are more predisposed to *Kalmia* invasion and subsequent reduced growth of black spruce seedlings than fertile sites. This would be consistent with our results because low soil fertility has been shown to increase condensed tannin concentrations in *Kalmia* leaves (Bradley *et al.*, 1997, 2000b), as plants shunt more carbon towards the synthesis of non-structural carbon compounds (Haukioja *et al.*, 1998; Koricheva *et al.*, 1998)

It is curious that acid phosphatase activity was repressed by tannins *in vitro*, but was unaffected by litter mixtures in the microcosm study, nor was it correlated to *Kalmia* cover in the field. Compared to the other two enzymes, acid phosphatase activity was relatively high, which indicates a high P deficiency before the addition of litter. Because acid phosphatase is an inducible enzyme (Sinsabaugh & Moorhead, 1994; Allison & Vitousek, 2005), it is possible that available P in the fresh litter suppressed its activity, which may have obscured any tannin effect. This explanation is consistent with the observed reduction in acid phosphatase activity that we observed in the litter-amended microcosms following 2 and 6 weeks incubation.

The *Kalmia*:black spruce leaf litter ratio used in Experiment 2 represented a realistic range of litter ratios occurring in the field, with *Kalmia* representing up to 90% of total annual leaf litterfall on sites approaching 50% *Kalmia* cover. We acknowledge that sampling litter over the course of a single growing season may lead to an overestimation of *Kalmia* litter relative to that of black spruce. While it is true that black spruce tends to lose needles year round, there is usually a peak in litter production in the fall (Bares & Wali, 1979); conversely, although *Kalmia* tends to produce litter in the fall, this evergreen shrub can still produce litter throughout the year (Titus *et al.*, 1995). More important to our study is the fact that *Kalmia* has a 2–3 year leaf-span whereas needle longevity for black spruce was estimated at 5-8 years by Vincent (1965) and at more than 10 years by others (e.g., Greenway *et al.*, 1992). Hence, for the same amount of standing leaf biomass, *Kalmia* will produce 4–5 times more annual leaf litter than black spruce. Furthermore, fine root litter production by *Kalmia* on cutover sites can be 2–3 orders of magnitude greater than by black spruce (Damman, 1971; Thiffault *et al.*, 2004); in a parallel study, we found similar chemistry and concentrations of tannins in *Kalmia* fine roots as in *Kalmia* senescent leaves (unpublished data). Given the importance of belowground biomass production and turnover by *Kalmia*, the amount of condensed tannins released in the soil on *Kalmia*-dominated cutovers may be far greater, therefore, than what we estimated from our litterfall data.

Ecological implications

There is an inherent limit to what can be extrapolated from *in vitro* studies, because litters of different species differ in more aspects than simply the quality and quantity of the tannins they contain. Here, we devised a three-tier experimental approach, with a microcosm litter-mixture experiment designed to link the reductionist approach used in Experiment 1 to field-level observations reported in Experiment 3. Throughout these three experiments, we observed consistent effects of tannins, *Kalmia* litter, and *Kalmia* cover on β-glucosidase

activity. This enzyme is particularly significant, because the hydrolysis products are important energy sources for soil microbes (Tabatabai, 1994). It is, therefore, consistent that we find organic matter content to be positively related to β-glucosidase activity (Fig. 4) in the field, and that soil microbial communities are increasingly energy deficient following the invasion of *Kalmia* and other ericaceous shrubs (Bradley *et al.*, 1997; Bradley *et al.*, 2000a). Furthermore, the cycling of litter N, perhaps the most important plant nutrient, is stoichiometric with the cycling of litter C (McGill & Cole, 1981), so that a concomitant reduction in N cycling is expected with a reduction in the activity of C-degrading enzymes such as β-glucosidase.

Whitham *et al.* (2006) have made a case in favour of "ecosystem genetics," by providing strong evidence linking leaf litter tannin production in different hybrids of cottonwood (*Populus spp.*) trees to important differences in ecosystem processes such as decomposition and soil N cycling. In their view, the fact that a genetically-based plant trait such as leaf litter tannin concentration leads to inter-specific plant interactions, illustrates the link between gene expression within one species and community-level (i.e., ecosystem-level) "phenotypes." In our study, black spruce and *Kalmia* tannins differed both quantitatively and qualitatively. We showed in Experiment 1 that both of these tannins inhibit important soil enzymes, and that these effects are concentration-dependent. Given that tannin concentrations are five times greater in *Kalmia* than in black spruce litter, the results of Experiments 2 and 3 represent a strong conjecture to support the notion that litter tannin production is an important trait in *Kalmia* driving ecosystem structure and processes so as to improve its competitive ability.

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CHAPITRE IV

EFFET DU TYPE DE COUVRE SOL ET EFFETS SPÉCIFIQUES DE L'ÉPINETTE NOIRE ET DE KALMIA SUR LES PROPRIÉTÉS DU SOL, LA DISPONIBILITÉ DES NUTRIMENTS ET LA DÉCOMPOSITION DE LA LITIÈRE.

Référence : Joanisse, G.D., Bradley, R.L. et C.M. Preston. Ground cover type and specific effects of black spruce and *Kalmia* vegetation on soil properties, availability of nutrients and litter decomposition (Plant Soil à soumettre)

La modification potentielle de la disponibilité des nutriments du sol par une plante devrait varier selon les conditions abiotiques (i.e., texture du sol, drainage et luminosité) et biotiques (i.e., les plantes accompagnatrices et leurs litière, le couvre sol) retrouvé sur un site, ainsi que les caractéristiques propres à l'espèce (i.e., profondeur des racines, durée de vie des tissus). Les concentrations en tanins semblent être importantes pour réguler les mécanismes décrits aux Chapitre I, II et III. Et la concentration des tanins dans les feuilles et racines varient avec les conditions du site. Sur un site avec moins de nutriments mais dont la photosynthèse n'est pas limitée, il va y avoir une accumulation de tanins condensés dans les feuilles. De même, si les nutriments sont disponibles, mais que la photosynthèse est limitante, la plante investira plus dans la production de protéines que de composés phénoliques. Nous avons effectivement démontré dans une autre expérience que l'augmentation d'ensoleillement entraîne une augmentation de concentrations de tanins condensés dans les feuilles de *Kalmia* (ANNEXE 1). De plus, des différences de couvre sols apporte des conditions micro-environnementales et nutritionnelles différentes qui influencent l'accumulation de la matière organique et sa composition chimique, ainsi que la décomposition des litières de plantes associées. Compte tenu que le *Kalmia* se retrouve sur des sites de différentes qualités et sous

la canopée forestière, il est envisageable que son effet sur la modification des nutriments du sol variera selon les sites. De ce fait, j'ai caractérisé la matière organique retrouvée sur différent sites (parterres de coupe de lichen et mousse, et forêts fermées) par ^{13}C CPMAS RMN, la disponibilité de l'azote et du carbone dans le sol sous des plants de *Kalmia* et d'épinette noires, ainsi que la décomposition de litière de *Kalmia*. Nos résultats indiquent qu'il y a des différences de la composition organique des sols retrouvés dans les différents sites, mais que peu importe les conditions de sites, le *Kalmia* diminue la disponibilité du carbone et de l'azote dans les sols, et que cette réduction se fait surtout dans l'horizon organique où la majorité des racines de *Kalmia* se retrouvent. Cependant, contrairement aux attentes, les concentrations d'azote organique dissous (DON) étaient inférieures dans les sols de *Kalmia* que dans les sols d'épinette, ce qui suggère encore une fois que la concentration en DON n'est pas nécessairement reliée à la concentration de tanins. Dans les horizons minéraux, la disponibilité du carbone était supérieure pour les sols d'épinette que de *Kalmia,* possiblement à cause de la présence de racines d'épinette dans ces horizons. De plus, la décomposition de litière a été réduite sous les épinettes dans les sites à lichen comparativement à ceux dans les sites à mousse, ce qui suggère une plus faible disponibilité des nutriments pour l'épinette dans les sites à lichen à cause d'une interaction négative entre le lichen et les racines d'épinette. Globalement, ces résultats indiquent que même dans la forêt fermée, quoique la litière de *Kalmia* soit de meilleures qualité que celle des parterres de coupe, le *Kalmia* diminue la disponibilité des nutriments relativement à l'épinette noire.

J'ai élaboré et réalisé les expériences de laboratoire et de terrain, ainsi que l'analyse des spectres RMN, et ma participation à la rédaction du manuscrit fut importante. Comme pour les autres manuscrits présentés, la mise en place des différentes parcelles d'études CPRS a été réalisée par l'équipe du Dr. Alison Munson et les parcelles d'études de forêts non-coupées ont été mise en place par Félix Boulanger (ancien étudiant à la maîtrise du Dr. Bill Shipley). Le Dr. Bill Parsons a également révisé l'orthographe et la grammaire anglaise d'une première

version de ce manuscrit. La rédaction du manuscrit a été réalisée avec la collaboration de mon directeur, Dr. Robert Bradley.

Abstract

Kalmia angustifolia is an ericaceous shrub that can rapidly spread following forest harvesting and cause a slowdown in soil nutrient cycling, especially that of N, through a top-down effect caused by the release of condensed tannins. We hypothesised that *Kalmia* would reduce litter decomposition, C and N availability and microbial activity, and increase soil tannin and DON concentrations, and that this top-down effect would be more pronounced in lichen than in moss sites, and within the forest floor than in the underlying mineral soil horizons. First, we compared, with ^{13}C-solid state NMR analysis, the organic carbon composition of forest floor material that developed in lichen-spruce and spruce moss cutovers, as well as in closed-canopy spruce-moss forests. NMR indicated that organic carbon composition is different for the two cutovers, depending on whether either lichen or moss tissues accumulated. Second, we compared the physico-biochemical soil properties of the forest floor, and those of the surface and subsurface mineral soil horizons, which developed under patches of *Kalmia vs.* patches of black spruce trees occurring on these different sites. *Kalmia* reduced soil mineral N and C availability in all site types compared to black spruce, and mostly in the forest floor, even if its litter was of superior quality in the closed canopy forest. Contrary to expectation, DON concentrations were lower in soil under *Kalmia* than under spruce, which we discuss in terms of protein-tannin complexes and the competitive ability of *Kalmia*. Third, we compared *Kalmia* litter decomposition in *Kalmia* and spruce patches on lichen and moss cutovers. Litter decomposition was similar between sites and patches, except that mass loss was lower in spruce patches in lichen sites than in *Kalmia* patches, which we interpret as inhibition of spruce mycorrhizae by lichens. Taken collectively, our data suggest that *Kalmia* reduces soil N and C availability relative to black spruce, and that forest harvesting in sites with a *Kalmia* understory will exacerbate N and C deficiency. Thus, our study indicates that *Kalmia* patches should be taken into account by foresters who plan to harvest timber in these forests.

Introduction

The development of "precision agriculture" attests to the importance of small-scale spatial variability in soil properties in controlling crop yields. This practice allows farmers to manage their fields according to localized needs identified by an intensive GPS-guided grid sampling (Zhang et al. 2002). Compared to agricultural fields, natural forests usually present a higher diversity of plant species and, accordingly, this is expected to result in a higher spatial variability of soil properties, both horizontally and in the vertical development of the soil profile. In the boreal forests of Quebec (Canada), the cycle of forest disturbance – usually wildfire or clearcutting – and secondary succession, further allows this spatial variability to change over time. In these forests, however, silvicultural practices such as stand thinning, clearcut harvesting, scarification, fertilization or slash-burning are applied uniformly throughout each forest management unit. Likewise, indices of soil quality (e.g. fertility, drainage, pH, depth, etc.), which are meant to guide silvicultural prescriptions, are measured on bulk samples that inform forest managers about the average soil quality values of each management unit. Given that these forests comprise few tree species, only the effect of the dominant tree species on soil properties is usually considered in the management decision process (Saucier et al. 1998). However, recent studies have shown that the understory vegetation comprises a substantial amount of biomass contributing to the cycling of soil nutrients (Wurzburger and Hendrick 2007; Messier and Kimmins 1991), and is a major driver of forest biogeochemistry and productivity (Nilsson and Wardle 2005). The understory vegetation in boreal forests is much more diverse and dynamic than overstory trees (Légaré et al. 2001), and a disregard for the localized effects of dominant understory plant species on soil properties at different successional stages may result in localized responses to silvicultural treatments that either meet or forestall forest management objectives. While "precision forestry" in Quebec's boreal forest is not foreseeable in the near future, it is nonetheless important to appreciate the localized effects of key understory species.

Black spruce (*Picea mariana* (B.S.P.) Mill.) is the most common boreal tree species in Quebec, and ericaceous shrubs such as *Kalmia angustifolia* L. are often a major component of the understory. *Kalmia* usually occurs in dense patches, and soil conditions in these patches are likely to be affected by *Kalmia* roots and litter inputs. *Kalmia* produces tissues that are rich in polyphenolic compounds such as tannins (Joanisse et al. 2007), which can be transferred to the soil via throughfall or litter inputs. It is thought that through the action of these compounds, *Kalmia* can interfere with black spruce regeneration (Titus 1995; Lebel et al., in press). One proposed mechanism that may account for this interference is the modification of soil nutrient availability, especially that of N. The formation of protein-tannin complexes is likely to be an avenue for limiting N mineralization because of their low degree of degradability and associated slow rates of N release (Hattenschwiler and Vitousek 2000; Howard and Howard 1993; Kraus et al. 2003; Bennett and Prescott 2004). Phenolic compounds are also alleged to reduce long term rates of litter decomposition (Kraus et al. 2003), which would influence carbon availability to soil microorganisms. Phenolics can also be readily leached from the litter to the soil where they can interfere with soil nutrient cycling. This leaching can also contribute to mass loss (Kraus et al 2003). Thus, we expect soils that have developed below patches dominated by *Kalmia* to be richer in phenolic compounds, to be lower in mineral N relative to dissolved organic nitrogen (DON), and to show lower microbial activity and slower litter decomposition rates than soils in adjacent patches occupied by black spruce seedlings.

Following a major stand disturbance, transmittance of incident light to the shrub layer is initially high but gradually falls as the overstory vegetation develops. High light intensity should result in a high production of carbon-based phenolic compounds, such as condensed tannins (Koricheva et al. 1998). Likewise, as overstory trees develop, their contribution to total soil litter inputs increases, and accordingly, so does soil nutrient availability (Bradley et al. 2000a). As predicted by the carbon/nutrient ratio hypothesis (Bryant et al. 1983), higher

soil nutrient availability is expected to result in plants shunting more non-structural carbon towards the synthesis of protein and less towards the synthesis of secondary metabolites. Thus, the potential effects of *Kalmia* on the abovementioned soil properties are likely to diminish during stand development, as the availability of light decreases and that of soil nutrients increases (Bradley et al. 2000a). These potential effects of Kalmia on soil properties may also vary as a function of the ground cover. In the boreal forest of Quebec, there are two main black spruce forest ecotypes, lichen-spruce open woodland occurring on dryer sites, and spruce-feather moss forests occurring on moist sites (Saucier et al. 2003). Lichen sites are expected to be less productive, and to show lower accumulation of soil organic matter and mineral N than moss sites (Sedia and Ehrenfeld 2003). It is, therefore, possible that *Kalmia* will have a proportionately greater effect on soil properties in lichen than in moss sites.

Kalmia establishes itself mainly through rhizomatous growth and its root system is very dense and localized (Hall et al. 1973; Titus et al. 1995). Studies have shown that the root system of *Kalmia*, like that of other ericaceous shrubs with similar life-history traits such as salal (*Gaultheria shallon* Pursh.), has a net preference for establishing itself and exploiting coarse woody debris and other partially decomposed substrates in the forest floor (Huffman et al. 1994). Black spruce, on the other hand, is known to germinate and spread its primary root system down into the mineral soil horizon before extending a fine-root system into the forest floor (Burns and Honkala 1990). We expect, therefore, to find a greater contrast in soil properties between the forest floor and underlying mineral soil horizons under *Kalmia* than under black spruce. Given that increasing light intensity favours the vegetative spread of *Kalmia*'s belowground structures (Inderjit and Mallik 2002), we expect this contrast to be greater during early successional stages than in a closed-canopy forest.

We report on a study where we first compared, with ^{13}C-solid state NMR analysis, the organic carbon composition of forest floor material developed in lichen-spruce and spruce

moss cutovers, as well as in closed canopy spruce-moss forests. Second, we compared the physico-biochemical soil properties of the forest floor, and those of the surface and subsurface mineral soil horizons, which developed under patches of *Kalmia* and patches of black spruce trees occurring on these different sites. We hypothesised that *Kalmia* would reduce C and N availability and microbial activity, and increase soil tannin and DON concentrations, and that this effect would be more pronounced in lichen than in moss sites, and in the forest floor than in the underlying mineral soil horizons. Third, we tested the hypothesis that *Kalmia* litter decomposition is faster in moss than in lichen ground cover, as well as within patches of spruce than those of *Kalmia*. The ecological implications of our results are discussed in terms of their relevance to forest management.

Material and methods

Field site descriptions

Our field sites were located in the boreal forest near the town of Senneterre, in the Abitibi region of Québec, Canada (ca. 48° N, 76° W). Soils of the region are classified mainly as Humo-Ferric Podzols (Blouin and Berger 2001). Mean annual temperature is 0.5 °C and the average annual precipitation is 972 mm (Environment Canada 2002). Plots (50 x 50 m) were established on 20 independent sites located within a 75 km radius. Sixteen sites had been harvested for black spruce 10 years previously; the other four sites were unharvested, closed canopy spruce-feather moss forest. Timber harvesting had been accomplished by "careful logging," which is referred to in Québec as CPRS (MRNFPQ 2003). The practice of CPRS (Cut with Protection of Regeneration and Soils) requires that heavy machinery traverse no more than 25% of the harvested area, that crowns and branches remain on site, and that advanced regeneration of black spruce be maintained. Eight of the 16 cutover sites occurred

on moist soils (mesic drainage class), where the ground cover consisted of a feathermoss mat, composed mainly of *Pleurozium schreberi* (Brid.) Mitt; the other eight cutover sites occurred on dry soils (xeric drainage class), where the ground was carpeted with fruticose lichens (*Cladonia* and *Cladina* spp., mainly *Cladina rangifera*). The four undisturbed forests were dominated by black spruce trees and had a canopy openness varying from 11.9 to 20.4 % (personal communication, F. Boulanger, average of 25 hemispherical photos, unpublished master thesis). The understory shrub community was composed of various ericaceous shrub species, including *Kalmia*, *Vaccinium* spp., *Rhododendron groenlandicum* (Oeder) Kron & Judd, and *Gaultheria procumbens* L. *Kalmia* dominated the shrub layer both in the undisturbed forest and in the cutovers.

Forest Carbon composition by ^{13}C CPMAS NMR

Forest floor composition of the moss- and lichen-dominated sites was compared using carbon-13 nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (^{13}C CPMAS NMR). Six of the 16 cutovers were randomly chosen (3 lichen and 3 moss), together with one site from uncut forest. Blocks (25 x 25 cm) of forest floor (F-H layer) material, 5-10 cm thick, were collected every 5 m along two 50 m transects established on each site. The soil blocks were pooled within site and sieved to pass a 5 mm mesh. The 7 bulked samples were transported on ice to the *Laboratoire d'écologie des sols – Université de Sherbrooke*, and stored at 4°C until analyses began two weeks later. Solid-state ^{13}C NMR spectra of the forest floor samples were obtained using a Bruker MSL 300 spectrometer (Bruker Instruments Inc., Karlsruhe, Germany) operating at 75.47 MHz. Finely-ground samples were spun at 4.7 kHz in 7-mm diameter zirconium oxide rotors. Spectra were acquired with a 1 ms contact time, 2 s recycle time and 8000 scans. Spectra were processed using a 30 Hz line-broadening and baseline correction in Win-NMR 6.0 (Bruker Instrument Inc., Germany). Chemical shifts are reported relative to tetramethylsilane (TMS) at 0 ppm,

with the reference frequency set using adamantane. The spectral divisions were based on previously published work (e.g., Lorenz et al. 2000; Preston et al. 2006) and were assigned on the basis of local minima of the spectra. The NMR spectra were divided into the following chemical shifts: 0 to 50 ppm attributed to alkyl C; 50 to 93 ppm to methoxyl, N-alkyl (amino acid) and O-alkyl C; 93 to 112 ppm to di-O-alkyl C; 112 to 140 ppm to aromatic C-C and C-H; 140 to 160 ppm, aromatic C-O and C-N; 160-187 ppm, carbonyl C (carboxyl, amide and ester C). The alkyl-C to O-alkyl-C ratio (0-50/50-112) was calculated as an index of the advancement of forest floor decomposition (Lorenz et al. 2000). Areas of specific shift regions were determined after integration and were expressed as the percent of the total area (relative intensity). Areas were not corrected for spinning sidebands, as they were relatively small, and their effects would be similar among most of the samples. There are limitations on the quantitative reliability of CPMAS spectra, but it is appropriate to use them to compare intensity distributions among similar samples (Preston 2001)

Effect of *Kalmia* and black spruce on physico-biochemical soil properties

Soil and vegetation sampling

On each site, the forest floor (F-H layer) and underlying mineral soil (Ae and B; 0-10 cm) were collected under six patches of *Kalmia* and six patches of black spruce at the end of June 2005. Patches were selected along crossed transects, were at least 5 m apart, and excluded (i.e., above ground) the other type of vegetation, i.e., black spruce patches were free of *Kalmia* plants (< 5%, visual determination) and *Kalmia* patches were free of black spruce trees. The Ae horizon material was grey- to tan-coloured, and overlaid darker brown B-horizon material. Soil samples from each site were combined by vegetation cover type (*Kalmia* versus black spruce) and sieved to pass a 5-mm mesh to remove coarse debris and

roots. The 120 bulked samples were transported on ice to the *Laboratoire d'écologie des sols – Université de Sherbrooke*, and stored at 4°C until analyses began the following week.

On October 2002, green and senescent *Kalmia* leaves were randomly collected along the transects, placed on ice and lyophilized before analysis. For spruce, we only collected green and senescent needles along transects in the cutovers. Along transects in the cutovers, we also collected 4-6 whole *Kalmia* plants, including associated soil, and brought them to the lab to collect fine roots (<1-mm diameter). The root systems were rinsed with distilled water to remove all soil debris. Fine roots were harvested and lyophilised before chemical analysis. A subsample of each vegetation was ground in a ball mill and analysed for total N following acid digestion, total C by high temperature combustion and thermoconductometric detection (Vario CN Analyser, Elementar GmbH, Germany), and condensed tannins and total phenolics, as described below. After analysis, intact *Kalmi*a senescent leaves from the lichen and moss cutovers were pooled together for the litter bag experiment (see below).

Soil analysis

Total N, P, organic matter content and pH

Total N and P contents of the forest floor material from each site were determined colorimetrically using a Technicon Autoanalyser (Pulse Instrumentation, Saskatoon, SK), following wet digestion of oven-dried and finely-ground subsamples. Soil pH was measured electrometrically on aqueous suspensions (soil:water = 1:10 F-H and 1:2 Min) of air-dried material. Organic matter content was estimated by loss-on-ignition (Nelson and Sommers 1996).

Dissolved N concentrations

Mineral N (NH_4^+ and NO_3^-) was determined by extracting fresh soil subsamples (7-15 g dry mass equiv F-H and Min (Ae and B), respectively) with 30 or 75 ml of 1.0 N KCl. Solutions were shaken for 1 h on a reciprocal shaker and vacuum-filtered through Whatman No. 5 cellulose papers; F-H extracts were subsequently filtered through a 0.45 μm low-protein binding syringe filter. Aliquots of the filtrates were analysed colorimetrically for NH_4^+-N (nitroprusside-hypochlorite-salycilate) and NO_3^--N (Cd reduction-sulphanilamide), using the Technicon Autoanalyser. The FH aliquots were also analysed for total dissolved N (TDN), using a modified persulphate oxidation technique (Cabrera and Beare 1993). Briefly, 10 ml of persulphate solution was added to 5 ml of extracts (in duplicate), and the sealed mixtures were autoclaved at 121°C for 45 min. The extracts were then analysed for NO_3^--N. Dissolved organic N (DON) concentrations were then calculated as the difference between inorganic-N (NH_4^+-N and NO_3^--N) and TDN concentrations. Potential mineral N availability was assessed by aerobic incubations of another set of fresh subsamples in the dark at 20 °C for 30 days, analysing inorganic-N and correcting for their initial concentrations (i.e., net N mineralization). In all cases, NO_3^--N concentrations were below detection limits of our instrument (0.06 ppm) and are not reported. Concentrations were corrected to an oven-dry mass basis, following gravimetric determinations of soil moisture content (105°C for 36 h).

Microbial activity and microbial biomass

Basal respiration (BR) rate was measured by placing fresh soil samples (ca. 7-15 g dry mass equiv for F-H and Min soils, respectively) into 125-ml plastic jars, flushing the headspace (5 min), sealing jars with air-tight lids equipped with rubber septa, and sampling aliquots of air in the headspace with a needle and syringe after 4 h. Headspace CO_2 concentrations were analysed with Micro-GC (Chrompack, Middleberg, The Netherlands). Ambient CO_2

concentration and temperature were regularly noted during the trials. For each sample, ambient CO_2 was subtracted from sampled CO_2 concentration and the difference was adjusted using Ideal Gas Laws to 22°C, assuming $Q_{10} = 2$. After the incubation, the lids were opened to aerate the sample. The following day, microbial biomass was measured on the same samples.

Microbial biomass (MB) was determined by substrate-induced respiration (SIR, Anderson and Domsch 1978). Samples previously weighed for BR were transferred to 500-ml plastic containers and amended with glucose (1000 μg C g^{-1} soil). The amendments were applied with talc as 500-mg mixtures and dispersed throughout each soil subsample using a kitchen handmixer with one beater. The amended subsamples were then transferred into 125 ml gas sampling jars and left uncovered for 100 min to reach optimum SIR rates. Each subsample was then flushed for 5 min with ambient air, sealed for 30 min, and headspace air was analysed for CO_2 concentration using a GC (as described above). SIR rates were converted to MB using equations described by Anderson and Domsch (1978).

Condensed tannins and total phenolics

Condensed tannins were measured colorimetrically using the proanthocyanidin assay (butanol-HCl hydrolysis), standardised against purified black spruce and *Kalmia* tannins for soil under black spruce and *Kalmia*, respectively (Preston 1999). Briefly, samples were lyophilised, ground in a mortar and pestle, and extracted twice with 70% aqueous acetone, which was then dried down under N_2 for the determination of extractable tannins. The insoluble residue was dried under N_2 for the analysis of residual tannins; butanol-HCl reagent was added directly to the residue. Total condensed tannins was calculated as the sum of extractable and residual tannins.

Total phenolics were determined after rehydrating 0.5 ml aliquots of dried acetone-water extracts with 1.0 ml distilled water, by adding 0.5 ml Folin-Ciocalteu reagent (Sigma) and 2.5 ml of aqueous Na_2CO_3 (20% w/v). Solution absorbance (750 nm) was read on a spectrophotometer standardised against tannic acid (Sigma-Aldrich) (Waterman and Mole 1994).

Leaf litter decomposition experiment

To test whether *Kalmia* leaf decomposition varied between Ecotype and Vegetation cover, we placed four litterbags under three black spruce and three *Kalmia* patches in 3 lichen and 3 feather moss cutovers (total of 144 litterbags). Litterbags (10 x 10 cm) were made of polyester mesh with an aperture size of 1 mm. Litterbags, which contained approximately 1 g of lyophilised senescent *Kalmia* leaves, were weighed precisely, and inserted into feathermoss and lichen layers under the different vegetation patches in May 2003. Three litterbags per vegetation cover per Ecotype were retrieved in August 2003, October 2003, May 2004 and June 2005 (after 3, 5, 12, 25 months). After retrieval, the litterbags were cleaned of debris and roots, lyophilised and weighed for mass loss calculation.

Statistical analysis

Qualitative comparisons of NMR spectra for the forest floor were made for all spectra according to the existing literature, while quantitative analyses were performed on lichen and moss cutover spectra by two different means. First, we compared the distribution of relative areas between moss and lichen cutovers using PERMANOVA v.1.6 (Anderson 2005), which is a permutation-based program for analysing multivariate data on the basis of any distance

measure (Anderson 2001; McArdle and Anderson 2001). To perform the multivariate analysis, we permuted the data 499 times with Euclidean distance as our similarity measure; due to our small sample sizes, the correct *P*-values (P_{MC}) were obtained through Monte Carlo random draws from the asymptotic permutation distribution (Anderson and Robinson 2003). Values for the relative areas of the 0-50 ppm region were removed prior to analysis, as they were the most similar between lichen and moss, and their inclusion would sum the variables to 100%. Following multivariate analysis, *t*-tests were done to compare each relative area of the selected regions between moss and lichen cutovers. As only one closed-canopy site soil was characterised, no statistical comparisons were made with the cutovers.

For comparing soil properties under black spruce and *Kalmia* in the different site types, we ran linear mixed-effects models (Pinheiro and Bates 2000) separately by soil horizon. Mixed-effects analysis of variance (Type-III ANOVA) included site (20 sites) as a random factor, while Ecotype (Closed canopy forest, Lichen cutovers, Moss cutovers) and Vegetation (*Kalmia*, black spruce) and their interactions were considered fixed effects. When no significant interactions were found, the mixed model was rerun without the interaction terms to analyse the main effects. When significant interactions were found between Ecotype and Vegetation, the mixed-effect model was run with type of vegetation or ecotype separately, and when significant effects where found, means of Ecotype were compared by Tukey's tests. Linear mixed-effect were analysed using the R statistical package (R Development Core Team 2005).

To gain an overall picture of the differences between the combinations of soil and vegetation, data for the soils were subjected to discriminant function analysis (DFA, discriminant procedure, method direct). DFA were done separately for each soil horizon, and variables that were highly correlated ($r>0.60$) were excluded from the analysis to limit collinearity. For FH, DFA was performed on all variables except residual tannins, which were highly correlated

with extractable tannins. For Mineral Ae soils, eight variables were used (Moisture, OM, pH, NH_4-N, net NH_4, BR, MB and Total phenolics) and for Mineral B soils, six variables where included (OM, pH, NH_4-N, net NH_4, BR and MB). One-way ANOVA was then used to compare each classified group for Function 1 and Function 2, followed by post-hoc Tukey's test. DFA and ANOVA where performed using SPSS 11.01 (SPSS Inc., Chicago, IL.).

To compare green leaf, litter and root chemical characteristics among Ecotypes and Vegetation, two-way ANOVA was performed separately for each tissue type. When significant effects were found, Tukey's post-hoc test was used to separate the means.

For percent mass remaining in the litterbags, a linear mixed-effect model was used as described above, with site as the random factor (6 sites), and Ground cover (Lichen, Moss), Vegetation (*Kalmia*, Spruce) and Months (3,5,12 and 25 months) and their interactions as fixed effects.

Prior to all analyses, we verified that the data conformed to the assumptions of normality and homogeneity of variance; data were ln-transformed when necessary to meet these assumptions. The level of significance for all tests was set to $P \leq 0.05$, unless specified.

Results

^{13}C-CPMAS NMR of forest floor of the different ecotypes

The interpretations of the NMR spectra were based on previous studies of litter and humus (Almendros et al. 2000; Kögel-Knabner 2002; Lorenz et al. 2000; Preston 2001; Preston et al. 2000). The spectra of the FH horizon from the three lichen and three moss cutovers, and closed canopy forest are displayed in Figure 1, and relative areas are given in Table 1. Spectra are very similar, and are also similar to those previously published for boreal forest floors (Hannam et al. 2004; Lorenz et al. 2000; Preston et al. 2006). The largest peak is at 73 ppm in the O-alkyl region, and is mainly due to carbohydrates (cellulose and hemicellulose). The sharp peak at 105 ppm is also mainly due to carbohydrates. The peak in the alkyl region (0-50 ppm) has two maxima, at 30 and 33 ppm, characteristic of CH_2 in long chains, while the underlying broader intensity is due to a variety of CH, CH_2 and CH_3 (methyl) structures. The peak at 33 ppm represents mainly accumulation of long chain CH_2 from cutin, suberin and plant waxes, although microbial biomass may also contribute in this region. The main differences are the presence of more defined and bigger peaks in the aromatic-C region of 112-140 ppm (130 ppm) for moss cutover relative to lichen and closed-canopy forest, and higher O-alkyl peak in lichen cutovers (Table 1). Carboxyl-, amide- and ester-C produce the peak at 174 ppm. This region is mainly associated with the amide-C of proteins and the carboxyl groups of microbial and plant lipids. The alkyl-C to O-alkyl-C ratio is highest in the closed canopy forest, followed by moss cutovers and lichen cutovers (Table 1). MANOVA revealed significant differences between the overall distribution of relative areas for lichen and moss cutovers ($F_{1,4}$ = 8.53, P_{MC}= 0.038). The *t*-tests revealed that relative areas were significantly higher in moss cutovers for the carbonyl (160-187 ppm) (P <0.05), while the relative area of lichen was higher in the O-alkyl region (50-92 ppm) (P <0.05, Table 1). The aromatic-C region was higher in moss than in lichen, but this difference was not statistically significant (P <0.10).

Modification of soil below *Kalmia* and Black spruce

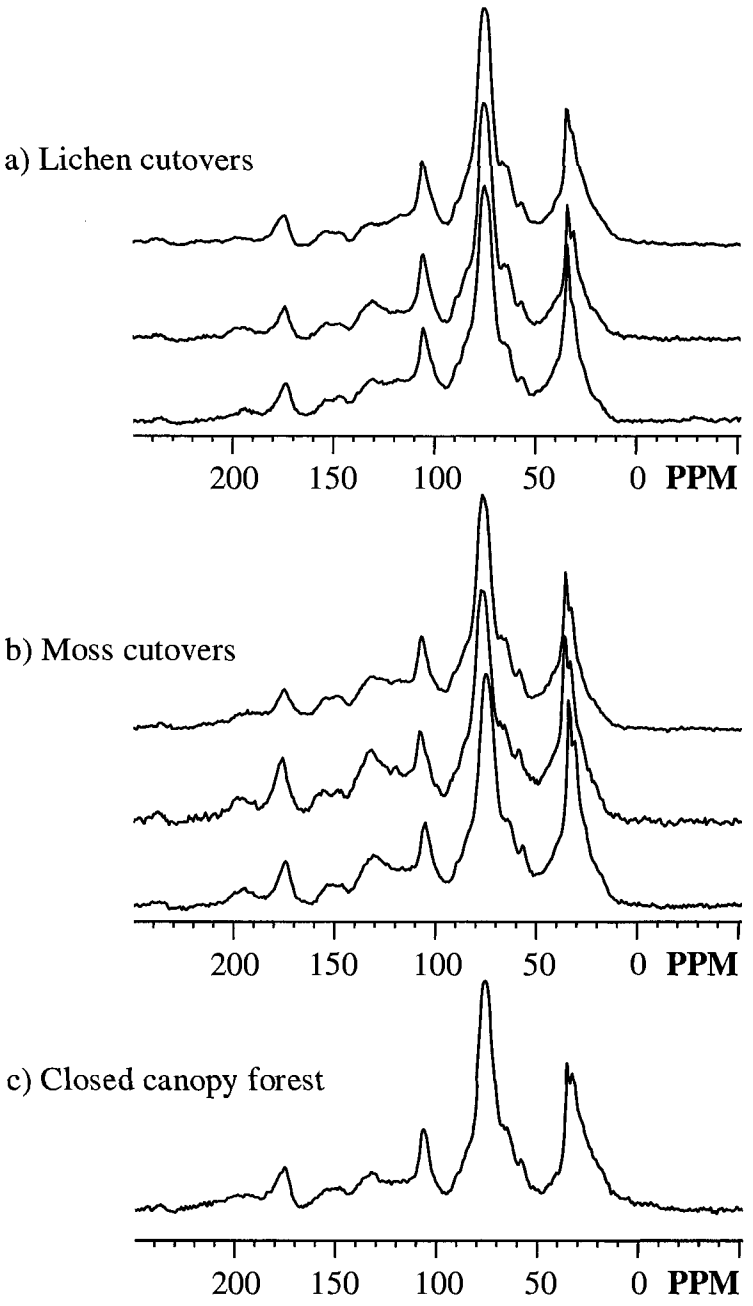

Figure 1. ^{13}C NMR spectra of forest floor (FH) originating from a) three lichen cutovers, b) three moss cutovers, and c) closed canopy forest.

Table 1. Relative intensities (percent of total area) of the ^{13}C CPMAS NMR spectra of organic layers originating from lichen and moss cutovers, and closed canopy forest.

	Range (ppm)						
	Alkyl-C	O-alkyl C		Aromatic C		Carbonyl C	
Ecotypes	(0-50)	methoxyl and O-alkyl C (50-92)	di-O-alkyl C (92-112)	C-C and C-H (112-140)	C-O and C-N (140-160)	carboxyl, amide and ester C (160-187)	Alkyl/ O-alkyl
Moss cutovers	24.5 (2.4)	41.8 (1.3)*	10.1 (0.9)	13.3 (0.7)°	4.4 (0.7)°	5.9 (0.8)*	0.47 (0.06)
Lichen cutovers	23.9 (1.7)	47.9 (2.9)*	11.2 (0.4)	10.1 (2.2)°	3.1 (0.8)°	3.9 (0.7)*	0.40 (0.02)
Closed canopy	27.4	43.4	8.7	10.3	4.6	5.5	0.53

Values are means of 3 sites for Lichen and Moss (1sd), and 1 site for Closed Canopy forest. * and ° indicates significant differences with $p<0.05$ and $p<0.10$ respectively following *t*-test of the Lichen and Moss Cutovers.

Forest floor FH horizon

For the forest floor, there were significant effects of Ecotype on pH, extractable N-NH_4, Net N mineralization and DON, significant effects of Vegetation on Moisture, organic matter, pH, extractable N-NH_4 and DON, and significant interactions between Ecotype and Vegetation for basal respiration and condensed tannins. More specifically, post-hoc analysis revealed that pH of moss cutovers was lower than pH of lichen cutovers and closed-canopy forest, and that pH, organic matter, microbial biomass (Fig 2. a,b,f) and moisture were higher under *Kalmia* (260% water content) than under Spruce (190% water content). Post-hoc analysis revealed that extractable N-NH_4, net N-NH_4 and DON were higher under closed canopy than either lichen or moss cutovers, and were higher under Spruce than under *Kalmia* (Figure 2 c,d,g). Exploration of the interactions revealed that basal respiration was significantly higher under Spruce than *Kalmia* in the closed canopy only (P =0.0445, Fig 2f). Furthermore, basal respiration was higher under *Kalmia* in closed canopy than in moss cutovers (P =0.0252), and higher under Spruce in closed canopy than either lichen or moss cutovers (P <0.001, Fig 2 e). While condensed tannins concentrations were higher under *Kalmia* than under Spruce for all Ecotypes (P <0.03), there were no significant differences between Ecotypes (P >0.10, Fig 2h). In FH soil, all other variables were not significant, but there was a tendency for higher total phenolic concentrations under *Kalmia* than under Spruce (4.6 vs 4.1 mg phenolics g^{-1} soil).

Mineral AE soils

For Mineral Ae soils, there were significant effects of Vegetation on total N, organic matter, extractable N-NH_4, basal respiration, and microbial biomass, a significant effect of Ecotype on Net N mineralization, and a significant interaction between Vegetation and Ecotype for

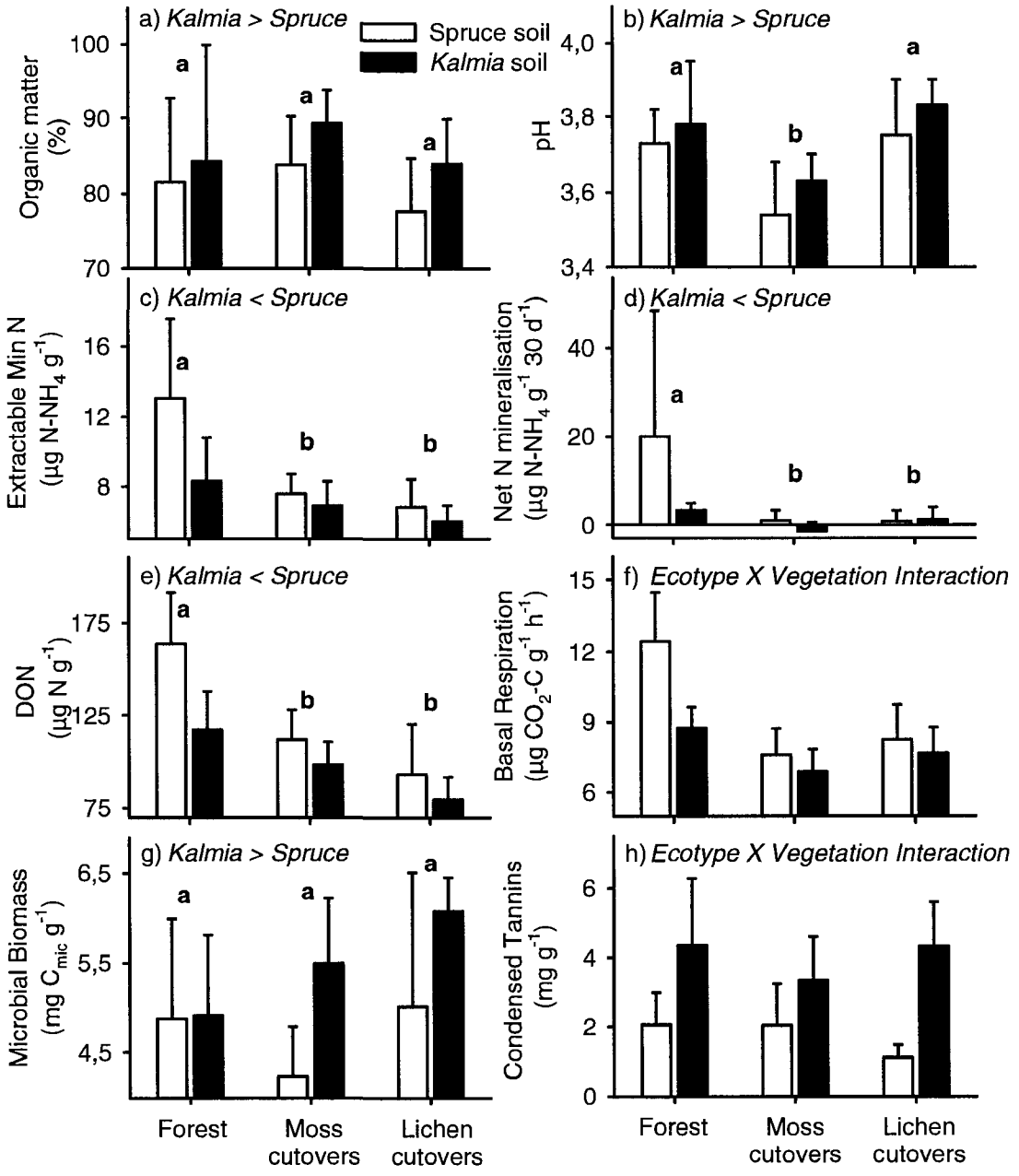

Figure 2. Mean of selected variables in the forest floor (FH) horizon under *Kalmia* and spruce in three ecotypes: closed-canopy forest (n=4), moss cutovers (n=8) and lichen cutovers (n=8). These are the untransformed original data. Bars = 1SD. Different lower case letters indicates significant differences between ecotypes, while significant differences between *Kalmia* and spruce are indicated on the top left of each frame ($p<0.05$).

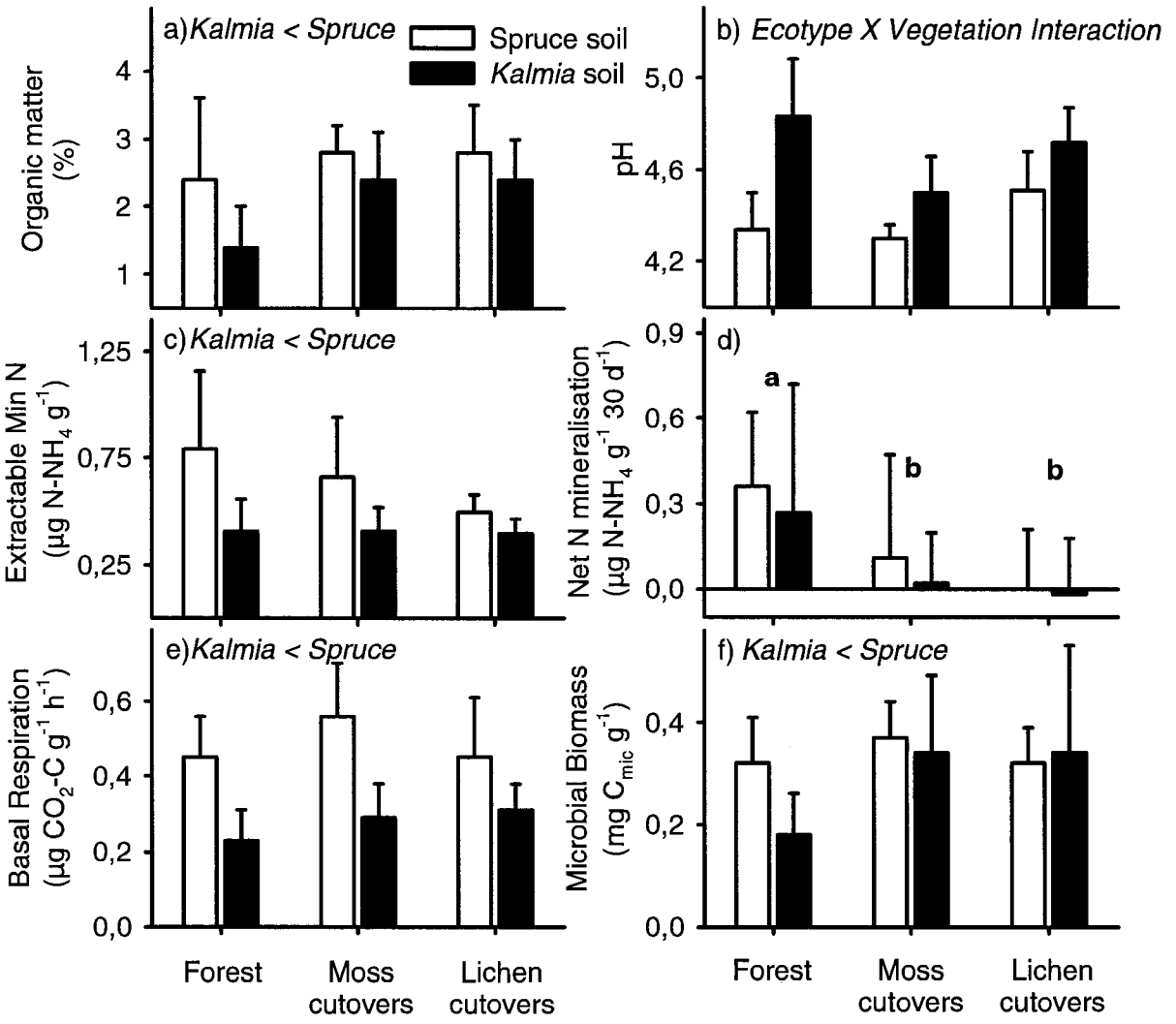

Figure 3. Mean of selected variables in the Mineral Ae horizon under *Kalmia* and spruce in three ecotypes: closed-canopy forest (n=4), moss cutovers (n=8) and lichen cutovers (n=8).. These are the untransformed original data. Bars = 1SD. Different lower case letters indicates significant differences between ecotypes, while significant differences between *Kalmia* and spruce are indicated on the top left of each frame ($p<0.05$).

pH. More specifically, organic matter, extractable N-NH_4, basal respiration, microbial biomass (Fig 3a,c,e,f) and Total N were higher in Min Ae soils under spruce than under *Kalmia* (For total N: 0.42 and 0.36 mg N g^{-1} soil under spruce and *Kalmia*, respectively). Net N mineralization was slightly higher in closed canopy forest Min Ae soils than in the cutovers (Fig 3d). Within each ecotype, pH of Min Ae under *Kalmia* was significantly higher than under black spruce (P <0.016); pH of Min Ae under spruce was higher in lichen than under moss ($F_{2,17}$=5.736, P =0.012), while pH of Min AE was higher under *Kalmia* in closed canopy sites than in moss cutovers ($F_{2,17}$= 5.571, =0.014) (Fig 3b). In Min Ae, differences for all other variables were not significant.

Mineral B soils

For Mineral B soils, there were significant effects of Ecotype on total N, moisture, organic matter, pH, N-NH_4, basal respiration and microbial biomass, and significant effects of Vegetation on pH and basal respiration. There were no significant interactions. More specifically, Total N, organic matter, moisture and microbial biomass were lower in lichen cutovers than both moss cutovers and closed-canopy, while basal respiration and N-NH_4 were lower in lichen than closed-canopy forest, and soil pH was higher in lichen than the other ecotypes (Fig 4 a,b,c,e,f, data not shown for total N and moisture). Basal respiration was higher under Spruce than under *Kalmia* (Fig 4e), while soil pH was higher under *Kalmia* than under spruce (Fig 4b). There were no other significant effects of vegetation and ecotypes on measured soil variables, but Net N mineralization is shown in Fig 4d).

Discriminant function analysis

For FH horizon, the first two discriminant functions explained 83.5 % of the variance. function 1 explained 55.3 % and DFA2 explained 28.2 % of the variance (Fig 5a). ANOVA and post-hoc analysis revealed clear separation in four different groups on Function 1 (P <0.001); spruce soil in the closed-canopy forest, spruce soil in lichen and moss cutovers, *Kalmia* soil in forest and moss cutover, and *Kalmia* soil in lichen cutovers (Fig 5a). Along the second axis, there is a separation by ecotype (Fig 5a). Overall, 95 % of the groups were correctly classified.

For Mineral Ae soils, function 1 explained 59.6% and groups were significantly separated (P <0.001), while function 2 explained 19.2 %, but groups were not significantly separated (P =0.058) (Fig 5b). One-way ANOVA revealed significant differences between group centroids for Function 1 and function 2. On function 1, there were three different groups; (1) spruce soil in moss cutover, (2) spruce soil in lichen cutover and *Kalmia* soil in moss cutover, and (3) *Kalmia* soil in forest and lichen cutovers. On function 2, spruce soil in the forest was different from all other soils except *Kalmia* soil in the forest (Fig 5b). Overall, 75 % of the groups were correctly classified.

For Mineral B soils, function 1 explained 65.8% and groups were significantly separated (P =0.008), while function 2 explained 20.2 %, but groups were not significantly separated (P =0.200) (Fig 5c). One-way ANOVA and post-hoc analysis between group centroids revealed that on Function 1, *Kalmia* soil in lichen ecotype was different from all others, and spruce soil in lichen ecotype was different than spruce soils in moss cutovers and forest, but not different than *Kalmia* soil in moss cutovers (Fig 5c). Overall, 62.5 % of the groups were correctly classified.

Variation of tissue concentrations among the different ecotypes

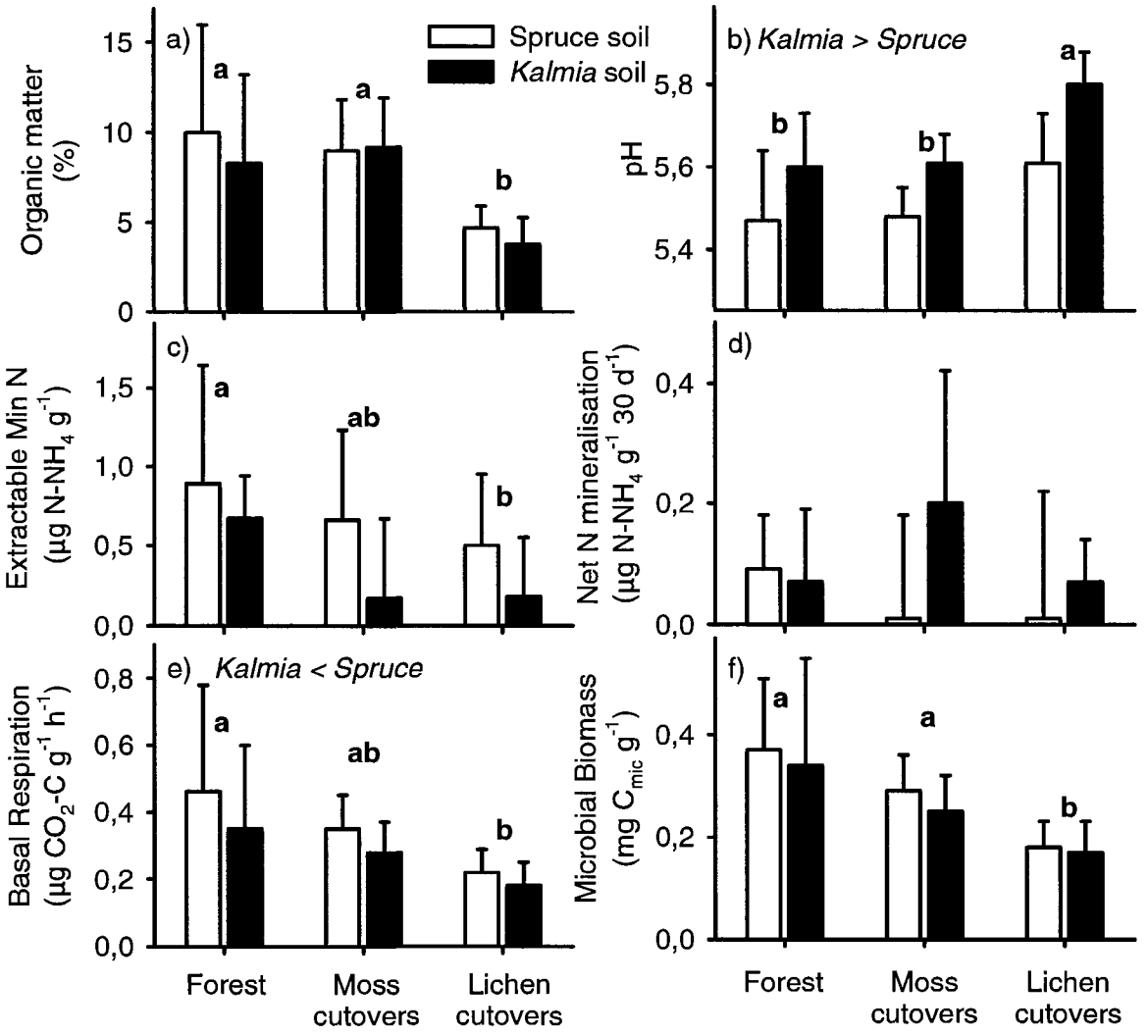

Figure 4. Mean of selected variables in the Mineral B horizon under *Kalmia* and spruce in three ecotypes: closed-canopy forest (n=4), moss cutovers (n=8) and lichen cutovers (n=8). These are the untransformed raw data. Bars = 1SD. Different lower case letters indicates significant differences between ecotypes, while significant differences between *Kalmia* and spruce are indicated on the top left of each frame ($p<0.05$).

There were significant differences between *Kalmia* leaves and spruce needles, both green and senescent, for most of the measured properties; there were few differences between

ecotypes (Table 2). *Kalmia* leaves, both green and senescent, had higher N, tannin and phenolic contents than spruce needles, and *Kalmia* senescent leaves had higher C content than spruce senescent needles. There were significant differences only for total phenolics and total N for *Kalmia* green leaves; total phenolic concentrations were higher in the cutovers than in the forest for *Kalmia,* while Total N was significantly higher in the forest than cutovers. There was a tendency for higher Total N in senescent *Kalmia* leaves in the forest (P =0.08). For spruce needles, only total phenolics in green needles were higher in the moss cutover than in the lichen. There were no significant differences between *Kalmia* roots from the moss and lichen cutovers for all measured variables.

Litterbag decomposition

The mean percentage of original litter mass remaining for litter bags in moss and lichen cutovers under *Kalmia* and Spruce vegetation is shown in Fig 6. For mass remaining, there were significant effects of Months, significant interaction between Vegetation and Months, and significant interaction between Ecotype, Vegetation and Months. There was a significant difference between treatments only at 25 months. There was a significant effect of Vegetation (P = 0.019) and significant interaction between Ecotype and Vegetation (P = 0.015). In the lichen ecotype, mass remaining was lower under *Kalmia* than under spruce (P = 0.007), and was lower in moss ecotype than lichen ecotype under spruce (P = 0.015, Fig 6).

Discussion

Effect of ecotype on Forest floor organic C: NMR spectra

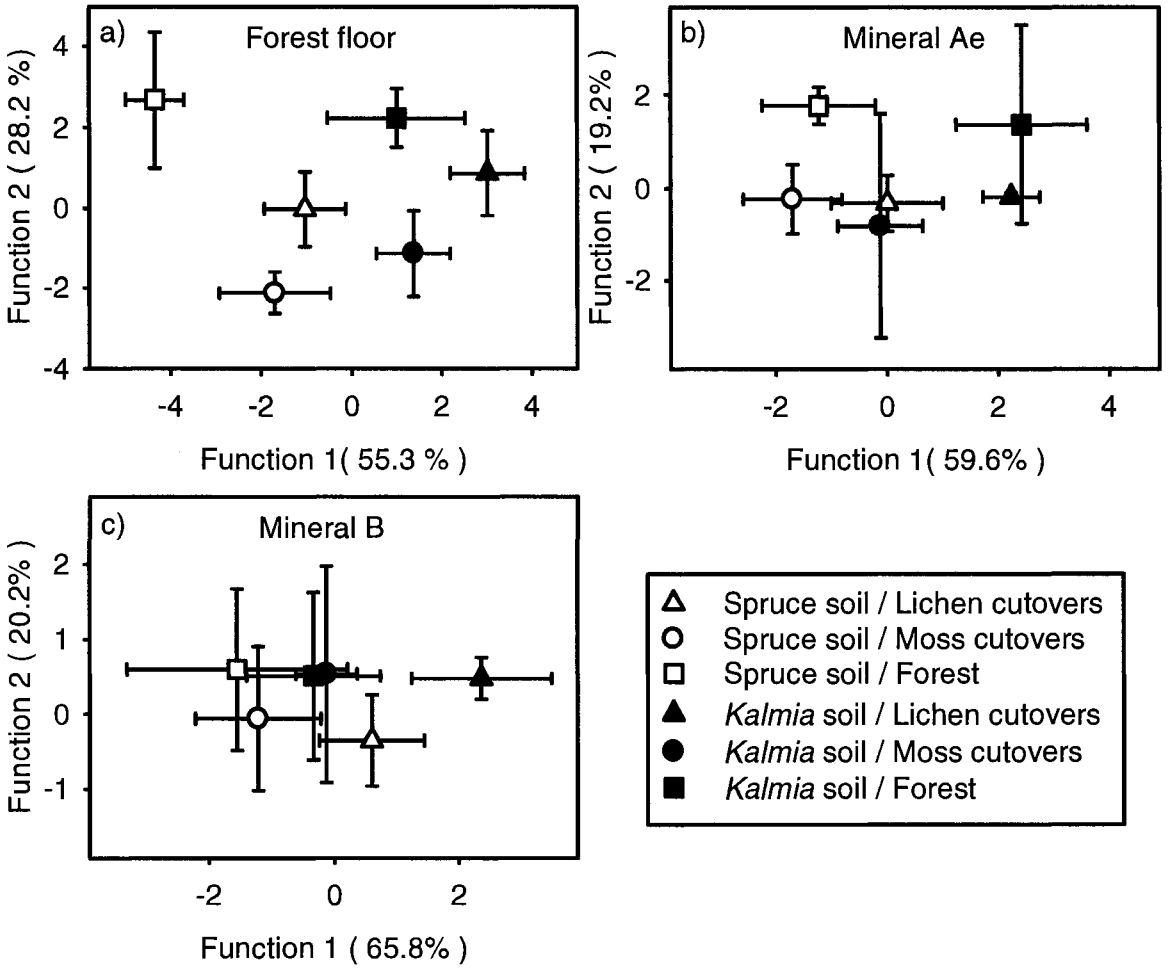

Figure 5. Ordination of the first two discriminant functions (DFA) of biochemical and biological variables for a) forest floor horizon (FH), b) Mineral Ae soils, and c) Mineral B soils (see text for detail). Each point represents the mean of 4 or 8 sites ± 1 SD for each function.

Lichen and moss forest floor appeared to exhibit similar chemical C structures, but the relative proportions of the different structures varied between lichen and moss cutovers (Table 1, Fig 1). This is probably related to the initial composition of lichen and moss ground cover, and also to the contribution of the vascular plants present on the site. Consistent with other studies, we found that FH soils under lichen was high in O-alkyl C (mainly

carbohydrates), and lower in aromatic- and phenolic-C (140-165 ppm) than in moss sites (Czimczik et al. 2003; Preston et al. 2006). This may indicate the dominance of lichen in the process of organic matter accumulation, as previous spectra of lichen have shown that they are very high in O-and di-O-alkyl-C, indicating mainly carbohydrate structure (Preston et al. 2006; Preston et al. 2000). Both lichen and moss tissues are low in aromatic C, but comparisons of lichen and moss spectra from the published literature indicate that aromatic-C and carbonyl-C regions are slightly higher in moss tissues compared to lichen tissues (Hannam et al. 2004; Preston et al. 2006). However, both cryptogams had small peaks, indicating the probable contribution of vascular plant tissues, such as roots, which can be rich in lignin and phenolic compounds (Preston et al. 2006). Furthermore, Sedia and Ehrenfeld (2003) have shown that oak seedlings growing in lichen patches had very fine white roots, whereas in moss patches, roots were bigger and of dark-brown colour. Although we did not compare root morphologies between our sites, our results suggest more lignified roots in moss cutovers that would then give higher aromatic signatures in the forest floor. Furthermore, higher aromatic C in the moss cutovers than in the closed-canopy forest indicates that forest floors from the cutovers have become more humified, which may reflect a reduction in vegetation inputs in the cutovers (Hannam et al. 2005). The higher ratio of alkyl-C to O-alkyl-C in the moss cutovers relative to the lichen indicates more decomposed organic matter, or possibly just differences in ratios of plants that contributed to the formation of the forest floor. Furthermore, the small differences between the spectra of cutovers relative to the different ecotypes suggest that past occupancy of black spruce is still detectable in the forest floor. Organic matter C composition (or relative distribution) is slightly different in the three ecotypes, which was corroborated by different Ecotype effects on soil biochemical differences in the FH (Fig 2,3,4,5).

Effect of vegetation on soil processes: *Kalmia* vs spruce

Table 2:Mean values of selected variables for *Kalmia* leaf, spruce needles and *Kalmia* roots across ecotypes.

		Variables			
Vegetation	Ecotype	Total N (mg g^{-1})	Total C (mg g^{-1})	Condensed tannins (mg g^{-1})	Total phenolics (mg g^{-1})
Kalmia green leaves	Lichen cutovers	10.2 (0.6) b	559.8 (5.3)	263.3 (12.7)	320.2 (5.7) a
	Moss cutovers	10.9 (0.8) b	555.2 (2.6)	272.5 (10.8)	332.1 (7.2) a
	Forest	13.2 (0.7) a	561.2 (3.4)	242.5 (28.3)	283.9 (25.0) b
Kalmia roots	Lichen cutovers	5.2 (0.5)	554.0 (6.8)	234.6 (22.1)	111.8 (9.2)
	Moss cutovers	5.4 (0.2)	557.3 (5.4)	238.8 (19.6)	130.3 (13.4)
Kalmia senescent leaves	Lichen cutovers	6.9 (0.9)	587.0 (6.6)	227.0 (25.9)	241.4 (12.3)
	Moss cutovers	6.5 (0.8)	573.6 (6.2)	222.7 (16.6)	225.8 (21.9)
	Forest	8.2 (0.5)	564.5 (0.2)	216.8 (19.9)	216.7 (18.9)
Spruce green needles	Lichen cutovers	7.2 (0.6)	529.5 (2.3)	93.9 (4.7)	100.7 (2.8) b
	Moss cutovers	7.0 (0.5)	536.3 (2.7)	103.2 (1.3)	107.9 (1.9) a
Spruce senescent needles	Lichen cutovers	2.3 (0.3)	580.0 (7.5)	48.5 (0.6)	96.0 (3.0)
	Moss cutovers	2.7 (0.4)	565.7 (2.0)	50.3 (1.9)	91.9 (1.9)

Mean (SE) of 4 for forest, or 8 for cutovers. When significant differences were within vegetation, means are separated by different lower case letters ($p<0.05$ following one-way ANOVA or t-test).

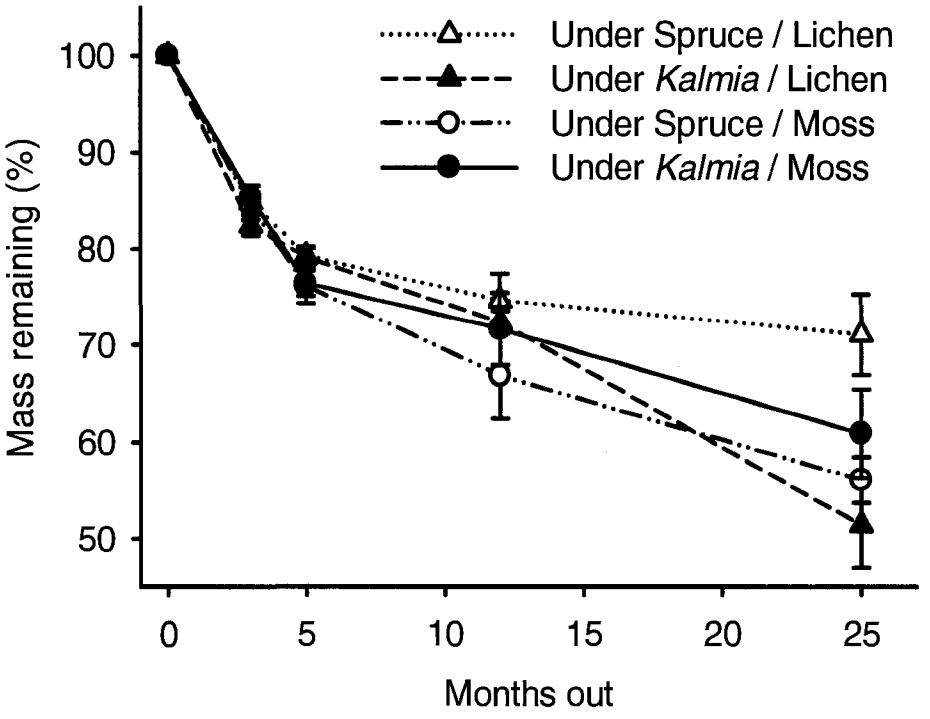

Figure 6. Percentage of *Kalmia* leaf litter mass remaining in litterbags during a 25 month incubation in the lichen and moss cutovers under *Kalmia* and black spruce. Means of 9 replicates ± 1 SD are shown for each point.

Overall, our results regarding the effects of *Kalmia* on soil nutrient availability and microbial activity of this study mostly agree with earlier reports (Bradley et al. 2000b; Inderjit and Mallik 1996; 1999) and demonstrate that sites with high *Kalmia* cover have lower soil nutrient availability (Damman 1971; Inderjit and Mallik 1999; Titus et al. 1995). Soils under *Kalmia* and under spruce were collected on the same sites, which enabled us to verify whether *Kalmia*-associated soils contained lower total nutrients or *Kalmia* modified the availability of nutrients by changing forest soil quality and microbial activity (i.e., a top-down effect). The hypothesis that *Kalmia* dominates poor micro-sites in this study can be rejected, because intrinsic forest soil properties, such as total N and P, did not vary significantly

between the two vegetation types. Only for the Mineral Ae soil was total N slightly lower under *Kalmia* than under spruce. From our results, it is clear that *Kalmia* reduces soil nutrient availability relative to black spruce, and that vegetation effects are not the same throughout the soil profile.

We more closely examined the FH horizon, where fine roots are more concentrated and where nutrient processes and microbial activity are very high compared to the mineral soil. Therefore, *Kalmia* should have the greatest negative effect on soil nutrient processes in this organic horizon. In fact, microbial respiration and microbial biomass was an order of magnitude higher in the FH than the underlying mineral soils (Fig 2,3,4). Moreover, *Kalmia* influenced biochemical and biological characteristics of the FH horizon. The higher moisture content of the FH horizon beneath *Kalmia* compared to black spruce has been observed elsewhere (Bradley et al. 1997), and might be explained by one of several possible mechanisms: canopy interception by spruce; a lower transpiration rate of *Kalmia* versus black spruce; a more water-use-efficient photosynthetic system or a higher photosynthetic rate per unit N for *Kalmia* (Small 1972); or simply a FH horizon that was richer in organic matter, thereby retaining more moisture. In fact, the slightly higher organic matter content in the FH under *Kalmia* than under spruce probably originates from higher fine-roots mass and material more recalcitrant to decomposition, since it has been shown that forests with high ericaceous cover accumulated more undecomposed organic matter (Krause 1998; Preston 1999), which is corroborated with higher tannin concentrations. Organic matter was slightly higher under *Kalmia* in the FH, but pH values were also slightly higher (Fig 2ab). This seems counter-intuitive, because many previous reports have mentioned a decrease in pH following ericaceous spread (i.e., Damman 1971). However, both coniferous and ericaceous vegetation can acidify soils (Raulund-Rasmussen and Vejre 1995), and both soils had very low pH values (Fig 2b). Other studies have shown slightly higher pH values in *Kalmia*-dominated sites compared to *Kalmia*-free sites (Inderjit and Mallik 1999), and this could be attributed to differences in litter quality. For example, Inderjit and Mallik (1996) have measured an

increase in forest floor pH originating from a mature black spruce-feather moss forest following amendment with *Kalmia* leaf litter. The exact mechanism for this response is unknown, but probably originated from different litter quality inputs or root exudates, since it is well documented that the pH of the upper soil horizon is partly a result of the composition of the green vegetation that produces this litter (e.g., Finzi et al. 1998; e.g., Swift et al. 1979; Wardle et al. 1997). However the differences were quite small (an increase of 0.1 pH unit), and is probably ecologically unimportant for the FH soil nutrient processes.

According to our prediction, there was a reduction in mineral N availability under *Kalmia* patches relative to black spruce patches. Lower mineral N availability in *Kalmia* soil was probably because of higher condensed tannin inputs than from spruce, as condensed tannins can reduce N mineralization and microbial activity (Bradley et al. 2000b; Kraus et al. 2003; add a Nierop et al. 2006a ref). This is consistent with higher condensed tannin concentrations measured in *Kalmia* FH soils than in spruce FH soils, and in *Kalmia* litter compared to spruce litter (Table 2). On cutovers, we found that there were 4.5 times more condensed tannins and 2.5 times more total phenolics in *Kalmia* leaf litter than in black spruce needle litter, which was consistent with a previous study (Joanisse et al. 2007). There were also high concentrations of condensed tannins in *Kalmia* fine roots, which suggest that these are released onto the soil and probably contribute to the high tannin concentrations measured in *Kalmia* soils.

Contrary to our hypothesis, DON in the FH horizon was higher under spruce than under *Kalmia* (Fig 2e). This seems contrary to previously reports, which speculated that an increase in polyphenols and tannins would be reflected in higher DON concentrations and decreased DIN concentrations in soil solution (i.e., Northup et al. 1995). An increase in DON relative to DIN concentrations is speculated to be the result of the formation of protein-tannin complexes that are recalcitrant to decomposition and subsequent mineralization (Howard and

Howard 1993; Northup et al. 1995). Recent studies demonstrate that tannins bind tightly to soil organic matter, and thus, protein-tannins complexes are not likely to be extractable by conventional methods (Lorenz and Preston 2002; Nierop and Verstraten 2006) as tannins can potentially form covalent interactions with soil organic matter (Hernes et al. 2001). Furthermore, Halvorson and Gonzalez (2008) have recently found that tannic acid additions reduced the amount of extractable soluble organic N in soil. The lower DON under *Kalmia* does not exclude the formation of protein-tannin complexes as an important mechanism of reduced N availability. Furthermore, it is also possible that a higher proportion of soil N, both mineral N and DON, is immobilized in the *Kalmia* root biomass and not available in the rhizosphere or bulk soil (Bloom and Mallik 2006; Thiffault et al. 2004).

The lower basal respiration in *Kalmia* FH soils, relative to black spruce FH soils, is consistent with the fact that *Kalmia* releases C-compounds of lower degradability. It has been shown that soil microbial communities are increasingly energy-deficient following the invasion of *Kalmia* and other ericaceous shrubs (Bradley et al. 1997; Bradley et al. 2000a). The higher microbial biomass measured by substrate-induced respiration might thus indicate that microbes in *Kalmia* soils are more C-deficient and respond more to the glucose amendment, relative to spruce soils. However, the higher microbial biomass may be simply related to higher fine root biomass in *Kalmia* soils compared to spruce soils, a situation that produces more soil microbes adapted at consuming complex organic nitrogen forms, such as protein-tannin complexes. However, the higher condensed tannins and lower basal respiration measured in *Kalmia* soils suggest that these soil organisms do not readily use tannins as a carbon source, indicating that metabolism of tannins is low or non-existant (Kraus et al. 2003).

Effects of *Kalmia* and black spruce on mineral soil nutrient processes were expected to differ from those observed in the FH horizon. In fact, vegetation effects were almost non-existent in

the deeper mineral B soils (Fig 4, 5c), and differences observed between *Kalmia* and spruce soils was probably due to different rooting depths. Ericaceous fine roots are found mostly in the organic soil layer, while black spruce can have more roots in the underlying mineral horizons (Damman 1971). Thus, higher organic matter, total N, mineral N and respiration rates in the mineral horizons under black spruce result from higher carbon inputs from spruce root exudates rather than inhibition by *Kalmia* (Fig 2,3,4), although recalcitrant carbon compounds can be released by the FH horizon of *Kalmia* soil and potentially leached into the mineral horizon where they can negatively affect microbial activity and N availability (Inderjit and Mallik 1996). However, our results indicate very low phenolic concentrations (<0.2 mg g^{-1}, data not shown) and no difference in phenolic concentration under *Kalmia* and black spruce in the mineral Ae soil. Nonetheless, the lower basal respiration found under *Kalmia* relative to black spruce in the mineral B horizon is consistent with lower carbon quality under *Kalmia*, since there were no differences between soil organic matter content (Fig 4). Furthermore, higher pH values under *Kalmia* than under spruce cannot be solely attributed to differences in cation composition and concentrations, as no great differences were observed in soil cation concentrations between *Kalmia*-infested and *Kalmia*-free closed canopy black spruce forest (Inderjit and Mallik 1999). In fact, Inderjit and Mallik (1999) even found lower Ca^{2+} concentrations in the mineral Ae horizon of *Kalmia*-infested soil than in the closed canopy forest, which suggests that other factors are responsible for decreased pH values under spruce, such as organic acids released from roots.

Effect of ground cover vegetation

Mosses and lichen differ in the ways in which they affect physical and biochemical properties (Cornelissen et al. 2007). For example, the high albedo effect of lichens reduces soil temperature. The two plant groups have different tissue compositions, growth rates and subsequent accumulations of organic matter. However, only few of the measured variables differed between moss and lichen cutovers for the FH; most differences occurred in the

underlying mineral soils (Fig 3 and 4). These differences included slightly lower DIN and DON concentrations in the lichen- than in moss-FH, but a higher pH in lichen- than moss-FH layers (Fig 2). These differences are probably linked to tissue quality, because it is known that moss can acidify soils (Cornelissen et al. 2007). With respect to the mineral B soils, there were consistent differences between lichen and moss, such as lower organic matter content, moisture, Total N, $N\text{-}NH_4$, basal respiration and microbial biomass, and higher pH (Fig 4). Since moss and lichens do not have roots, the differences between the effects of the two ground covers might be due to differences in past disturbance and differences in intrinsic site properties.

Although we do not have data for past disturbance on these sites, such as fire frequency and intensity, together with stand density before harvesting, differences in the mineral layer of lichen and moss soils could be the result of past fire disturbances. In northern latitudes, some spruce-lichen woodlands can originate from spruce-moss forests after intense fires have consumed the forest floor (e.g., Payette et al. 2000; Payette and Delwaide 2003), thus changing the soil cation availability. In a second study, we found that mineral Ae and mineral B soils from lichen cutovers had higher concentrations of total Ca, Mg and K than soils from moss cutovers (Joanisse, unpublished data), corroborating the higher pH values found in this study in lichen soils relative to moss soils. Furthermore, other characteristics, such as drainage class and related soil texture can favour establishment of moss over lichen, and mineral B soils of these lichen sites have coarser textures than mineral B soils of moss sites (W.J. Parsons, personal communication).

Mosses have been shown to contribute more to soil organic matter build-up than lichens (Sedia and Ehrenfeld 2003). Although we do not have specific data regarding organic horizon depth and bulk density under *Kalmia* and spruce on these sites, we do have data on average depth and density of FH horizon on these sites following random coring (W.J. Parsons,

unpublished data). Results indicate that moss cutovers have generally higher depths of FH than lichen soils (7.5 cm versus 6.2 cm), but with similar bulk densities, which suggest that on an area basis, there is more FH material in the moss cutovers than in the lichen cutovers. The greater amount of FH in moss cutovers should contribute to more total nutrients available for plant growth, and thus higher nutrient concentrations should be found in plant tissues on moss cutovers. However, our data indicate that there were no significant difference between lichen and moss cutovers for most of the leaf, root and litter measured variables (Table 2), which was reinforced by the fact that similar differences were found when comparing the effect of vegetation on the two different cutovers.

Effect of forest harvesting

Cutovers generally had lower mineral N, DON and basal respiration than closed-canopy forest. Following forest harvesting, a short-term increase in N mineralization and microbial activity is often measured, because of the carbon flush from decaying roots and of higher temperatures. As our cutovers were 10 years of age, probably any nutrient flush that occurred after forest harvesting either was gone or had been absorbed by the growing vegetation, thus giving lower mineral N values than in the closed canopy forest (Fig 2). The higher DIN and DON in closed canopy forest relative to cutovers have also been measured elsewhere (Bradley et al. 2000a; Hannam and Prescott 2003; Horner et al. 1987). Fine roots of ericaceous shrubs are more abundant after harvesting than in old growth, closed canopy forests, while the inverse was observed for black spruce fine roots (Smith et al. 2000). This higher fine-root mass could explain the increased organic matter content in FH soil found under *Kalmia* in cutovers and higher microbial biomass compared to spruce. Basal respiration, which is an index of available-C, was lower in cutovers than in uncut forest, which might be due to lower litter quality (Bloom and Mallik 2006), litter quantity or to lower C-inputs from roots (Bradley et al. 2001). The lower pH found in the moss cutover than

in the closed canopy forest might be due to higher microbial decomposition of the organic matter in the moss cutover relative to the forest, which liberated more organic acids (Chang et al. 1995) Overall, forest harvesting in these sites exacerbated C-deficiencies by reducing basal respiration and subsequent N mineralization.

Interaction effect of vegetation and ground cover vegetation on soil nutrients

Contrary to our hypothesis, the effect of *Kalmia* on soil nutrients and microbial activity was not enhanced in lichen sites compared to moss sites, as reflected by the relative absence of interactions among the singly-tested variables. However, the discriminant function analysis shows that the *Kalmia* lichen soil, especially the FH horizon, was separated not only from the other *Kalmia* soils, but also from the spruce soils, indicating enhanced global effects of *Kalmia* on soil processes in lichen-dominated sites that are additive (Fig 4). As expected, there were fewer differences between mineral soil types than between forest floors. Furthermore, the effect of vegetation and groundcover decreased with depth, as correctly-classified data decreased in mineral soils relative to the forest floor.

Higher tissue quality is related to higher N and C availability

The hypothesis that *Kalmia* tissues should be of higher quality in the closed-canopy forest was partly fulfilled; green leaves from the forest had higher N concentrations and lower phenolic compounds than leaves from the cutovers (Table 2). Small differences were observed for *Kalmia* leaf litter, with total N being slightly higher under the forest canopy than in the cutovers. However, litter decomposition and nutrient release is not only related to the tannin or N concentrations, but also to the tannin:N ratios found in litter (Driebe and

Whitham 2000) and polyphenol:N ratios (Mafongoya et al. 1998); litter with higher ratios decomposed less rapidly and released less N than litter with lower ratios. Thus, the higher N availability in *Kalmia* closed-canopy forest floor than in the cutovers could be due, in part, to the lower tannin:N ratio in the forest (26:1), compared to the cutovers (33-34:1). This result suggested higher C quality litter in the forest, which was reflected in higher soil respiration than in the cutovers (Fig 2f). However, even if *Kalmia* litter was of slightly higher quality in the forest, *Kalmia* still negatively affected nutrient availability relative to black spruce, as reflected in the lower microbial activity and lower N availability (Fig 1,2,3). Other studies have also found that ericaceous shrubs growing under shade conditions produced leaf tissues containing greater N and lower phenolic and condensed tannins concentrations (Iason and Hester 1993). Unfortunately, we did not have information for black spruce roots or needles in the closed forest, but spruce litter quality should also decrease as stand openness increases. Other studies have found similar, higher or lower phenolic concentrations in conifer foliage than in roots (Gallet and Lebreton 1995 ; Kraus et al. 2004). If we suppose as *status quo*, that black spruce roots contain the same concentrations of condensed tannins and phenolics as its needles, then there is still less tannin in spruce roots than *Kalmia* roots. However, it is not only tannin concentrations that are important, but also total allocation and production of the different tissues containing the tannins. Although we did not quantify the biomass of *Kalmia* in the different ecotypes, it has been shown that the biomass of leaves and roots produced by ericaceous shrubs under closed canopy decreased substantially compared to sunlight conditions, and that leaf-to-fine-root mass ratios greatly increased under shade conditions (Hawkins and Henry 2004; Messier 1992), indicating lower root production under a closed canopy. For example, if we suppose higher root density in cutovers than in a closed canopy forest for *Kalmia* roots, this would inevitably result in lower condensed tannin input in the closed-canopy forest floor. We did not measure such a difference, but from our unpublished data from another experiment (Joanisse et al., unpublished data), we found that *Kalmia* fine roots that came from shaded plants (16% light) contained 25% more condensed tannins than fine roots of *Kalmia* plants growing in the open (100% light). Further study on the allocation

of condensed tannin production in the different *Kalmia* tissues along environmental gradients should be done.

Interactive effects of vegetation cover and litter decomposition

Generally, a greater availability of nutrients in the surrounding environment has been shown to accelerate litter decomposition (e.g., Liu et al. 2006), we expected that mass loss would be greater in moss sites than in lichen sites, and greater under spruce vegetation than under *Kalmia* vegetation (Wardle et al. 2003). However, our results indicated that there was an interaction between vascular plant cover and ground cover; mass loss was higher in moss than in lichen under spruce only, and mass loss was lower under spruce than under *Kalmia* in lichen sites (Fig 6). This result is contrary to other studies, which found higher mass loss under moss than under lichen (Sedia and Ehrenfeld 2006), but mass loss can also vary with the vascular vegetation present. The lichen thallus contains a number of aromatic compounds, including phenolics, that have demonstrated antibacterial and antifungal activity in the lab, but not necessarily to soil microbes in the field (Stark and Hyvarinen 2003; Stark et al. 2007). Although we do not have information about root density and mycorrhizal infection of spruce and *Kalmia* growing on the different site types, Sedia and Ehrenfeld (2003) have measured lower infection by ectomycorrhizal (ECM) fungi in soils dominated by lichens than soils dominated by mosses, but no effects on ericoid mycobionts, the types that colonize *Kalmia* roots. Markkola et al. (2002) have also measured lower root quality and lower ECM diversity on pine roots growing in lichen mats. Several studies have shown ECM inhibition by lichen extracts in pure cultures (Brown and Mikola 1974; Goldner et al. 1986). Since mycorrhiza have been implicated in decomposition and nutrient translocation (Bending and Read 1995; Read and Perez-Moreno 2003; Zhu and Ehrenfeld 1996), lower litter mass loss in lichen sites could be due to lower mycorrhizal infection of spruce roots compared to moss sites, where

roots with mycorrhiza have been shown to proliferate in the decaying moss layer (Carleton and Read 1991; Read et al. 2004).

Ecological implications and forest management

This study demonstrates that, despite ecotype (lichen vs. moss) or the presence of disturbance (cutovers vs. closed-canopy forest), patches of *Kalmia* have a negative top-down effect on soil N and C availability relative to soils associated with black spruce. However, contrary to our hypothesis, we measured lower DON concentrations in *Kalmia* soils relative to black spruce soils, even though the *Kalmia* forest floor had higher concentrations of condensed tannins. Since black spruce is known to take up some forms of DON from the soil, a decrease in DON concentrations could result from more recalcitrant organic matter (i.e., non-soluble protein-tannin complexes) accumulating under *Kalmia*, which can increase soil N deficiencies for spruce. We posit that this decrease in DON in *Kalmia* soil can cause a positive feedback for *Kalmia*, as it is able to more efficiently take up recalcitrant N from its own forest floor than black spruce (Bradley et al., 1997). Further study is needed to validate this hypothesis. As we expected, *Kalmia* leaf litter was of higher quality in the closed-canopy forest, but negative effects in *Kalmia*-associated soils were still evident, contrary to another study that concluded that *Kalmia* effects on soil nutrient availability in closed-canopy forest were minimal (Bloom and Mallik 2006). Furthermore, results from our litter-decomposition study suggest that black spruce trees in lichen cutovers that receive *Kalmia* leaf litter from surrounding patches would probably be more nutrient deficient than in moss cutovers because of lower rates of leaf decay. Therefore, as *Kalmia* patches increase on the different sites, it is expected that soil N availability would decrease.

From the viewpoint of the forester, it is not currently cost-effective to measure the distribution of ericaceous patches found on a site, but if some ericaceous plants are found in the understory before forest harvesting, it should be taken into account that soil nutrient availability is already lowered in these patches. Soils under *Kalmia* vegetation do not necessarily reflect the actual site potential, as nutrients such as N (and potentially others, such P) are rendered less available (Nierop et al. 2006a; Nierop et al. 2006b). Furthermore, it has been shown that spruce growth is reduced when *Kalmia* plants are in close proximity to seedlings (<1 m) (Yamasaki et al. 1998), so an increase in *Kalmia* patchiness on a site will substantially decrease soil nutrient availability and spruce growth. As silvicultural treatments in closed-canopy forest are not practical, those prescriptions that readily reduce *Kalmia* vigor and spread should be done immediately after forest harvesting on sites that have *Kalmia* patches. Thus, mechanical site preparation, such as scarification, will be necessary to limit the spread of *Kalmia* patches which would otherwise decrease site potential (Thiffault and Jobidon 2006; Thiffault et al. 2004).

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CONCLUSION

Le premier objectif de mon projet était de déterminer si la formation de complexes protéines-tanins par le *Kalmia* était un mécanisme efficace de séquestration de l'azote qui lui procurerait un avantage vis-à-vis l'épinette noire. Cette étude a permis de démontrer que les tanins de *Kalmia* et d'épinette noire ainsi que des extraits de feuilles pouvaient former des complexes protéines-tanins, mais que par unité de masse de tanin, le *Kalmia* précipitait plus de protéines. De plus, une étude en microcosme a démontré que, peu importe la source, les tanins et complexes protéines-tanins étaient très peu minéralisés, qu'il y avait très peu d'azote relâchée des complexes protéines-tanins, et qu'il n'y avait pas d'immobilisation du N dans les microorganismes relativement aux protéines non-complexées. Ces résultats indiquent donc que les organismes du sol n'utilisaient pas ces substrats comme sources d'énergie et d'azote. De plus, une autre expérience a démontré que les mycorhizes communément retrouvés sur les racines de l'épinette noire, même s'ils n'étaient pas inhibés par les tanins, n'étaient pas capables de croître sur des complexes protéines-tanins relativement à des mycorhizes communément retrouvés sur le *Kalmia*. Globalement, nos résultats suggèrent que la présence de *Kalmia* sur des sites augmentera la quantité d'azote séquestrée sous forme de complexes protéines-tanins à cause des fortes concentrations de tanins condensés, et ceci lui conférerai un avantage, puisque ces mycorhizes sont capables de les dégrader, du moins en laboratoire. Des recherches futures sont nécessaires pour confirmer que le *Kalmia* est effectivement plus efficace que l'épinette noire pour acquérir de l'azote des complexes protéines-tanins en conditions naturelles.

Dans le deuxième chapitre, contrairement au concept proposé, j'ai démontré que le ratio DON :DIN n'augmentait pas de façon consistante avec la proportion de litière riche en tanins. Cependant, mes résultats suggèrent que la formation de complexe protéines-tanins a bien lieu

dans des litières riches en tanins. Ceci suggèrent donc que le ratio DON : DIN n'est pas un bon indicateur de succession de plante riche en composés phénoliques dans la forêt boréale.

Dans le troisième chapitre, l'objectif était de déterminer si une réduction de la disponibilité des nutriments et de l'activité microbienne sur des sites envahis par le *Kalmia* était due à l'inhibition des exo-enzymes du sol. Cette étude a permis de démontrer qu'autant les tanins purifiés de *Kalmia* que ceux d'épinette noire pouvaient inhiber l'activité des enzymes du sol, et que l'inhibition enzymatique augmentait avec la concentration de tanins. De plus, compte tenue que le *Kalmia* contient environ cinq fois plus de tanins par masse sèche dans ces feuilles que l'épinette noire, une augmentation de l'apport de litière de *Kalmia* par rapport à celle d'épinette noire dans de l'humus en laboratoire a entraîné une baisse de l'activité de deux enzymes, soit la β-glucosidase et l'amidase. Ensuite, un relevé au champ a permis de démontrer que l'activité de la β-glucosidase, une enzyme dont les produits d'hydrolyse procurent des sources d'énergie aux microorganismes, était négativement corrélée avec le recouvrement de *Kalmia*, indiquant alors une baisse énergétique pour les microorganismes du sol. Les résultats de ce chapitre démontrent donc un lien entre la forte concentration de tanins produits par le *Kalmia* et la réduction de la disponibilité des nutriments par l'inhibition des enzymes du sol.

Le quatrième objectif était de déterminer si l'influence du *Kalmia* sur la disponibilité des nutriments du sol variait selon le type de recouvrement au sol et la perturbation. De façon générale, même s'il y avait des différences entre les types de sites, peu importe le site où il se trouvait, le *Kalmia* diminuait considérablement les disponibilités de l'azote et du carbone par rapport à l'épinette noire. Par contre, les disponibilités de l'azote et du carbone étaient un peu plus élevées dans les sols de *Kalmia* sous la canopée forestière que dans ceux des parterres de coupe, et ce à cause d'une litière de qualité supérieure ou d'un apport moins important de racines. Nous avons également mesuré une plus faible concentration de DON dans les sols de

Kalmia que dans les sols d'épinette noire, même si des concentrations supérieures en tanin étaient mesurées dans le sol de ces premiers. Nous attribuons cela à la formation de matière organique récalcitrante et de complexes protéines-tanins liés à la matière organique du sol, mais de futures études sont nécessaires pour le démontrer. De plus, nous avons démontré que la décomposition de litière de *Kalmia* était réduite sous des épinettes dans les sites à lichen, ce qui laissait suggérer une inhibition par le lichen envers les mycorhizes des épinettes, mais ceci reste à démontrer.

De façon globale, les résultats obtenus au cours de mes travaux de recherche indiquent que la baisse de disponibilité des nutriments sur les sites envahis par le *Kalmia* est bel et bien dû, du moins en partie, à ces fortes concentrations de tanins condensés retrouvées dans ses tissus qui peuvent former des complexes protéines-tanins et inhiber les enzymes du sol. Certaines questions sont ressorties de cette thèse, tel que le lien entre les tanins et les formes d'azote disponibles dans le sol. Considérant l'importance du DON dans les écosystèmes forestiers boréaux, il faudrait caractériser les différentes formes présentes, mesurer les formes puisées par les plantes ainsi que leurs dynamiques dans ces sols. De plus, d'un point de vue évolutif, il reste à démontrer que les tanins relâchés par le *Kalmia* ne nuisent pas à son acquisition des ressources, mais augmentent son habilité compétitive et subséquemment, sa fitness.

Quoique nos études soient de niveaux fondamentales, elles nous permettent de faire un lien avec les pratiques sylvicoles actuelles au Québec, ainsi que de proposer certaines mises en garde. Premièrement, compte tenu que le *Kalmia* diminue la disponibilité des nutriments et produit un humus récalcitrant, toute forme de pratique sylvicole qui ne perturbe pas suffisamment le sol, comme une CPRS, risque de favoriser sa reproduction végétative et d'appauvrir davantage le site. Deuxièmement, des pratiques sylvicoles ouvrants partiellement la canopée devraient être évitées sur les sites avec du *Kalmia*, puisque déjà sous la canopée, il diminue la disponibilité des nutriments. Et troisièmement, si des mesures d'éradications du

Kalmia sont prévues, il restera à déterminer s'il y a rémanence des effets de ce dernier sur la disponibilité des nutriments de l'humus, même en son absence.

ANNEXE 1

CHANGEMENT DE LA COMPOSITION PHÉNOLIQUE DES TISSUS DE *KALMIA ANGUSTIFOLIA* SOUS DIFFÉRENTS RÉGIMES DE LUMIÈRE ET DE FERTILISATION

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Les concentrations et la production de composés phénoliques et de tanins condensés varient en fonction des conditions environnementales. Dans cette annexe, je présente une partie des résultats d'une expérience complémentaire dans laquelle je comparais les concentrations de polyphénols totaux, de tanins condensés et de composés phénoliques spécifiques retrouvés dans les feuilles et racines de *Kalmia* poussant dans différentes conditions environnementale, soit trois intensité lumineuse et deux niveaux de fertilisation. En gros, les résultats indiquent une augmentation des composés phénoliques totaux et des tanins condensés dans les feuilles de plants poussant à pleine lumière relativement à des plants poussant à l'ombre. Alors que la relation inverse est observée pour les racines. Pour les composés phénoliques spécifiques, certains augmentent alors que d'autres diminuent en fonction de l'intensité lumineuse et de la fertilisation. Le manque d'interaction entre la lumière et la fertilisation suggère, pour le *Kalmia*, qu'ils sont indépendants pour contrôler la production de composés phénoliques. Des recherches futures devraient déterminer les effets de ces composés phénoliques qui étaient affectés par la lumière et la disponibilité des nutriments sur les propriétés des sols. Plus

spécifiquement, les recherches futures devraient permettre d'établir une relation entre les pratiques sylvicoles qui modifient la lumière et la disponibilité des nutriments dans le sous-bois, le relâchement de composés phénoliques par *Kalmia*, et l'habilité compétitive de l'épinette noire

Pour cette expérience, j'ai utilisé le dispositif expérimental du Dr. Bill Shipley en Abitibi. Les étudiants Mesmin Kiki et Mathieu Dufresne m'ont aidé sur le terrain et au laboratoire. J'ai extrait et analysé les tanins et passé les composés phénoliques sur le HPLC. La Dr. Christiane Gallet a identifié les différents acides phénoliques. La rédaction du présent communiqué a été réalisée en collaboration avec mon directeur Dr. Robert L. Bradley et la Dr. Christiane Gallet. Un manuscrit officiel contenant tous les résultats de cette étude est en cours de préparation et sera écrit conjointement avec le Dr. Bill Shipley.

Abstract

Phenolic compounds of *Kalmia angustifolia*, an invasive ericaceous shrub, have been related to its ability to limit growth of neighbouring species and to modify soil properties in the boreal forest. The influence of light and nutrient availability on phenolic compounds (both monomeric and tannin fractions) in *Kalmia* leaves and roots were studied in a field experiment lasting three years. For the leaves, a high light intensity promoted the synthesis of total phenols, condensed tannins and some phenolic acids, whereas fertilisation reduced total phenols and had both positive and negative effects on various phenolic acids. For the roots, increasing light intensity reduced condensed tannins and increased the synthesis of some phenolic acids, whereas fertilisation had no effect on all phenolic compounds. Phenolic content of roots and leaves varied independently from one another. There were no significant interactions between light and fertilisation suggesting, therefore, that the effect of fertilisation is independent from that of light.

Introduction

Kalmia angustifolia is an ericaceous shrub commonly found in the understory of mature conifer forests in Eastern Canadian boreal regions. Following disturbance, *Kalmia* can spread rapidly through rhizomatous growth and maintain dominance over conifer seedlings. The high phenolic content of *Kalmia* litter, which has been linked to the modification of soil properties [1,2], has been proposed as an important factor responsible for this dominance. Given that environmental factors can affect the internal allocation of resources within plants [3,4,5], we tested the hypotheses that increasing light, or decreasing nutrient, availability would increase the concentration of phenolic compounds in *Kalmia* roots and leaves. The

long term objective of this study is to devise forest management strategies that manipulate the availability of resources to reduce *Kalmia*-induced conifer seedling growth check.

Materials and methods

The study site, located near the Town of Senneterre in the Abitibi region of Québec (48°43'N, 76° 29'W, 427 m a.s.l.), was formerly a lichen–black spruce forest that was clearcut harvested 10 y prior to the study. *Kalmia* cover was approximately 25% at the time. The experimental design consisted of a completely randomized block design comprising 15 shade frames giving three light intensities (16, 40 and 100 %), with or without annual fertilisation (NPK 20-7-10 (720 kg N/ha)). Following the third growing season, green leaves and fine roots (<5 mm diameter) were collected, freeze-dried, weighed and ground. This material was extracted with acetone:water (70:30), rotor-evaporated, and subsequent analyses were performed on the aqueous fraction. Total phenols were determined with the Folin-Ciocalteau reagent with commercial-grade tannic acid as the standard. Condensed tannins were determined with the butanol-HCl reagent and purified *Kalmia* condensed tannins as the standard.

To measure monomeric phenolic compounds, the extracts were hydrolysed (HCL 2N) at 95 ^{o}C for 60 min and analysed by HPLC using a μBondapak RP C18 column with a 0–20% (acetic acid:acetonitrile (0.5%)) gradient in 0.5% acetic acid solution with a flow rate of 1.5 mL/min. Identification/quantification of individual phenolic acids was performed by comparison of retention times with standard compounds. Unknown phenolic peaks were quantified relative to their peak area. The effects of light and nutrient availability on phenolic compounds were analysed with mixed-model ANOVAs using the R statistical package [6,7] with shade frames as random factors.

Results and discussion

For leaves, condensed tannin concentration was higher at 100% (155 mg/g) than at 16–40 % (141 mg/g) light intensity, but was not affected by fertilisation. Total phenolic concentration also increased with light intensity (352 mg/g at 40–100% vs. 298 mg/g at 16%), and decreased with fertilisation. For fine roots, total phenolic concentration did not vary with light intensity, but condensed tannins were significantly higher at 16 % (59 mg/g) than at 40–100 % (51–47 mg/g) of incident light.

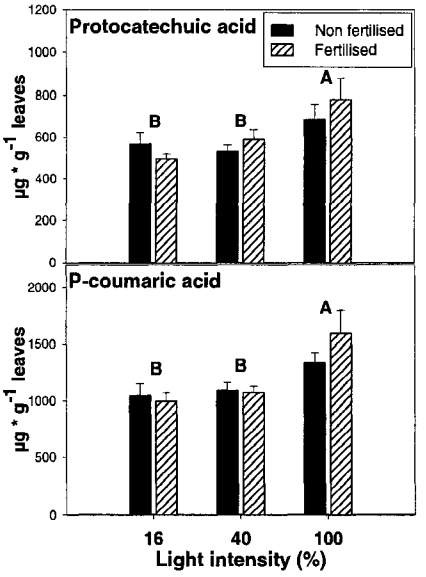

Figure 1. Concentrations of phenolic acids in green leaves ($\mu g*g^{-1}$; means ± S.E.) in response to light intensity and fertilisation. Different letters indicate significant differences between light intensities.

HPLC analyses revealed a number of known and unknown phenolic peaks that either increased or decreased in concentration with fertilisation in green leaves. For leaves, only p-coumaric and protocatechuic acid concentrations were higher at 100% than at 40–16% light intensity (Fig 1), while for other unknown compounds, no effect of light was found. No

significant effects of fertilisation were found on phenolic concentration in roots. Increasing light intensity increased relative concentration of some unknown phenolic compounds in roots, while others, such as P-coumaric acid, decreased in concentration (48 μg/g at 16% vs.34 μg/g at 100%).

There were no significant interactions between light and fertilisation, suggesting that the effect of fertilisation on *Kalmia* phenolic concentrations is independent from that of light effects. Phenolic compounds in leaves and roots were not correlated, suggesting that these two components respond independently to environmental factors.

In conclusion, the production of phenolic compounds by *Kalmia* may respond predictably to environmental changes. Forest management practices could use fertilisation as a means to limit the input of phenolic compounds to soil on *Kalmia*-dominated clearcuts.

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